

NITROGEN MINERALIZATION AND NON-SYMBIOTIC NITROGEN FIXATION
ON AN AND REQUIRED OF SLASH PINE PLANTATIONS IN NORTH FLORIDA

BY

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Abstract of Dissertation Presented to the Graduate School
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**NITROGEN MINERALIZATION AND NON-SYMBIOTIC NITROGEN FIXATION
IN AN AGE SUCCESSION OF SLASH PINE PLANTATIONS IN NORTH FLORIDA**

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Ammonification, nitrification, and non-symbiotic N_2 fixation were studied in an age sequence of slash pine plantations (0-, 5-, and 20-years-old) in North Florida to determine the effects of burning and of phosphorus and nitrogen fertilization.

Ammonification and nitrification rates were determined by incubating surface soil cores in the field and laboratory. In the laboratory, desaturated (aerobic) and saturated (anaerobic) cores were incubated at 25° C for 28 days. In the field, cores were enclosed with nylon-mesh bags containing a mixed-bed ion exchange resin to improve gas diffusion and prevent moisture content changes during the 28-day in situ incubation. Biological N_2 fixation was assessed using the acetylene reduction technique.

Results indicated little differences in ammonification and nitrification rates among plantation ages, except in the 20-year-old plantation site where ammonification was lower in the summer, and in the youngest site where nitrification was higher in the spring.

Average nitrification and denitrification rates were estimated to be 5-30 and 0.5 mg N/kg-so, respectively. In general, $\text{NH}_4\text{-N}$ concentrations were 25 mg/kg and $\text{NO}_3\text{-N}$ concentrations 2 mg/kg in the cores after the incubation period. Rates and concentrations in the field decreased from summer to spring.

Treatments had a small and temporary effect on the N processes, except in the N-fertilized plot where both nitrification and denitrification were highly stimulated. Potential N_2 fluxes rates were 3.5 kg/ha-yr and could account for about 10% of the N uptake of the vegetation. Treatments had little effect on fluxes, although consistently lower rates occurred in the N-fertilized sites. The recent cleared area showed higher rates, probably related to higher soil surface moisture contents, temperatures and light intensities.

A synthesis of information indicates that N-mineralization is the major source of available Thoracic N for vegetation uptake in established plants.

PRODUCTION

Over 110 million cubic meters of round wood are harvested annually from pine forests of the southeastern U.S. This represents about 40% of the total U.S. softwood fiber production (U.S. Forest Service, 1990). By the year 2025 it is expected that this figure will increase to 175 million cubic meters.

Intensive management practices are and will continue to be required to meet actual and projected demands, particularly if the forested land base decreases in extent. In order to achieve this goal, careful management operations, based on a knowledge of ecosystem processes, are essential to maintain high productivity levels and avoid site deterioration and declines in subsequent harvest returns (Ivimey, 1989; Petrichetti and Kuehn, 1992).

Forest stands of the lower coastal plain region of the southern U.S. usually grow on very infertile, poorly drained, acidic sandy soils. As such, these stands must depend to a large degree on nutrients coming in the precipitation, those stored in the forest floor and released through mineralization or fires, and on their ability to efficiently utilize and internally recycle nutrients (Miller et al., 1979). In the case of N, additional inputs may come through biological nitrogen fixation such as that by Factoria gracilis L. when present in the understorey of these forests (Pomeroy and Fisher, 1992).

Many studies have been performed in Florida to evaluate the effects of harvest methods, site preparation, burning, and fertilization on nutrient budgets, pine survival and growth, water quality, site deterioration, etc. [see, for example, Boffa et al., 1979; Gropper, 1979; Roberts, 1981; Morris, 1981; Coleman et al., 1980; Boffa and Fisher, 1982, and Boffa, Fisher and Arfsten, in press].

Nitrogen and phosphorus are the two most widespread essential soil nutrients limiting productivity of these forests as determined by fertilization trials. Large stem growth responses have occurred after application of either P or N or both to young or semi-mature pine plantations [Ballard and Arfsten, 1979; Fisher and Garbutt, 1980; Arfsten and Coleman, 1981; Boffa and Fisher, in press].

Forest nitrogen processes have been studied with some detail in Florida. For example, Boyer (1976) investigated nitrogen cycles of several southern Florida ecosystems using a mass balance approach. Boyer (1976) and Morris (1981) determined the effects of silvicultural practices on N mineralization and nitrification in recent clearcut areas, young pine plantations, and natural second growth stands. Neal (1971) and Thomas (1978) conducted P and N fertilization experiments to study their effect on soil chemical movement, denitrification, $\text{NH}_4\text{-N}$ volatilization, and nitrification. Perry (1981) and Boffa, Perry, Gropper, and Hensley (in press) investigated N and P mineralization and immobilization rates in decomposing slash pine needles.

Except for the work of Boffa, Perry, Gropper, and Hensley (in press) and Perry (1980) done using an age sequence of plantations, other studies have not been performed for full rotation periods. In

addition, there is little or no information regarding the potential R contribution of non-symbiotic biological fixation on these sites, and the possible interactions between P fertilization and prescribed burning on these and other nitrogen cycle processes.

Therefore, the general objective of the present investigation was to study the effects of nitrogen and phosphorus fertilization and prescribed burning on some nitrogen cycle processes on an age sequence of slash pine (*Pinus elliotii* var. *elliotii*) Lophocarpus plantation having similar site characteristics and silvicultural management conditions. The sites studied include a recently harvested 20-year-old plantation site, a 5-year-old plantation, and a 30-year-old plantation.

Several hypotheses were formulated:

- 1) P fertilization will stimulate nitrification, nitritification, and non-symbiotic biological fixation. As suggested by Cole and Ball (1981), N and P cycles should be closely interrelated due the fact that N processes tend to require large quantities of energy, with P in the form of ATP needed. Therefore, a shortage of P will restrict N cycle processes.
- 2) N fertilization will have a different effect on nitrogen processes. Although N mineralization will be stimulated by reducing C/N ratios and possibly by reducing toxin production (Galleher et al.) by the vegetation (Clark, 1980), non-symbiotic biological fixation will be inhibited.
- 3) Burning will stimulate nitrification, nitritification, and non-symbiotic biological fixation. During the fire, release of nutrients accumulated in the forest floor (especially P),

Increases in pH, and possible degradation of tannins are expected to occur, which in turn will stimulate N processes.

- 4) Effects of treatments on nitrogen mineralization will be different³ depending on plantation age. For example, older plantations tend to experience more severe shortages of available nutrients (Shelf, Fisher, and Fritzsche, in press). These conditions may negatively affect the microbially mediated N processes. In a tree that has not been harvested for many years, a larger release of accumulated forest floor nutrients is expected to occur after a fire. In addition, Elze and Pothofsky (1988) have hypothesized that old stands may produce tannins that will inhibit nitrification. In contrast, younger stands should not be limited in the same manner as the old plantation, although relatively low levels of nutrients still exist (Shelf, Fisher and Fritzsche, in press).

In a clearcut area, changes in microclimatic conditions, increases in organic matter in the soil, reduced vegetation nutrient uptake, and other factors created by the disturbance, are expected to have a significant impact on soil N processes (see, for example, Retzer and Vitousek, 1981).

LITERATURE REVIEW

Nitrogen Cycle in Forest Ecosystems

Fluxes and Storage

Nitrogen cycling is a very complex process because it includes microbially and chemically mediated transformations, various nitrogen-use species with different behaviors, and may be interrelated with other nutrient cycles, such as P. Transformations occur mainly between soil and atmospheric nitrogen as weathering processes are usually considered insignificant: during rock formation, elevated temperatures tend to volatilize nitrogen (Echerington, 1982).

A global view of the nitrogen cycle is presented in Fig. 1. Except for atmospheric and industrial fixation estimates, the remaining values are a little more than an order of magnitude accurate (Echerington, 1982), because of the relatively high degree of difficulty involved in working with storage and fluxes...

The nitrogen cycle is characterized by having a complex soil based component of very small magnitudes, and a very large but fractionally simple abiotic component (see Fig. 1). Both storages are relatively interconnected mainly through the microbially mediated processes of biological nitrogen fixation and denitrification.

Nitrogen dynamics and distribution for selected naturalized ecosystems are summarized in Tables 1 and 2. Total uptake is defined as all the nitrogen absorbed annually by the vegetation and net uptake as the N that stays in the vegetation, not recycled by litterfall.

Table 1. Nitrogen dynamics in selected unfertilized forest ecosystems (from Gentry, 1982)

System	Uptake		Return		
	Total	Net	Through-fall	Litter	Root-decomposition
by 8/ha-yr					
Eastern deciduous	74.8	8.0	8.3	88.3	2.1
Western deciduous	124.0	34.6	4.4	94.0	75.1
lobloche pine	88.7	71.1	3.2	27.8	28.9
shortleaf pine	117.1	5.6	6.8	58.2	66.7
Black spruce	---	28.8	---	---	---
Sub-alpine coniferous	39.1	13.8	1.3	18.3	---
Pandanus pine	---	6.5	---	---	---
Scots pine	38.8	23.0	---	---	---
Engelmann fir	---	9.12	---	7.98	---

Table 2: Nitrogen distribution (kg/ha) in some unfertilized forest sites. (from Kemp, 1988)

Component	Forest type						
	East-ern de-ciduous	East-ern de-ciduous	Loblolly pine	Sub-alpine conif-erous	Douglas fir	Aspen-birch	Jack pine
	kg/ha						
Tree	263	346	197	172	266	192	172
Stems	181	184	64	—	33	83	38
Forest floor	1185	774	283	656	176	79	261
Soil (0-60 cm)	3600	5686	1763	1588	2621	5000	1180
Sum	6229	6906	2304	1817	3156	6004	1651

throughfall or internal redistribution (increase in biomass N). In Table 3, average nitrogen contents and fluxes are presented for two natural forested forests in North Florida. Great variability occurs among and within ecosystems due to differences in climate and fertility conditions, to stand age, and to variations in sampling and analytical techniques used.

Core and Kopp (1982) have attempted to summarize systems observed in various worldwide temperate forest ecosystems, and concluded that:

- 1) Coniferous forest species and their nutrients have longer turnover times than deciduous forests.
- 2) On the average, only 10% of the nitrogen is held in above-ground vegetation storages.
- 3) Rates of nitrogen uptake and requirements are usually higher in deciduous than coniferous forests.
- 4) Coniferous forest trees internally retranslocate higher amounts of nitrogen than deciduous forests.
- 5) In coniferous forests, uptake and requirements of nitrogen strongly correlate with biomass production.
- 6) Coniferous species are more efficient than deciduous species in producing biomass with the same amount of nitrogen uptake.
- 7) Efficiency of production/unit nitrogen uptake increases as nitrogen becomes limiting.
- 8) In general, more nitrogen is added to forest ecosystem than is lost.

Table 2. Average nitrogen storages and flows for two flatland forests in North Florida (from Pritchett and Morris, 1982)

Components	N
<hr/>	
kg/ha	
<hr/>	
Reserves	
Soil logs and branches	44.0
Soilwood with bark	44.0
Understory veg.	26.0
Forest floor	27.0
Mineral soil (1 m)	1052.0
Total	1193.0
<hr/>	
kg/ha	
<hr/>	
Inputs	
Atmosphere	5.6
R. fixation	2.4
<hr/>	
Outputs	
Leaching	0.1
Evolution/Desuff	1.9
Desuff/Fixation	0.6
<hr/>	

In the southeastern U.S., Saloner and Saloner (2012) investigated nitrogen accumulation and cycling in loblolly pine (*Pinus taeda* L.) plantations in North Carolina during the first 28 years of development. Among their findings were that the amount of N in the system increased about 4 times when compared with initial nutrient storage, that a sizeable portion (about 20%) of the annual requirements was met by internal transfer, and that N tended to accumulate in the tree biomass and forest floor over time. They also concluded that the relative supply of N from the soil (soil) and organic sources may be low compared with the tree requirements, but biomass production was not yet inhibited due to the increasing redistribution of N within the trees. They suggested that one explanation for the relatively long-term observed response to fertilization in these stands, especially to P, was this ability of the stands to recycle nutrients internally.

A similar approach was used by Dale and Fisher (2002) and Dale, Fisher, and Pritchett (in press) when they investigated an age sequence of slash pine plantations in Florida. In these studies, changes in storages and flows of N and other nutrients were monitored in stands ranging from 2- to 20-years-old. Their conclusions included the following:

- 1) Maximum N uptake occurred between 2 and 10 years.
- 2) Very high N uptake rates were observed even in 2-year-old stands due to an active understory, with rates increasing less than 50% to the nucleus at 4-10 years.
- 3) Internal redistribution (trees and understory) increased greatly as the stands got older, due to the increasing dominance of pine.

- c) N and P use efficiencies (not primary production/assimilation) increased with time and were very high in the older plantations.
- d) N and P concentrations in tree components, including coarse roots, declined over time.
- e) Decreased substrate availability, especially of P, explained why there was a cessation in negative biomass increments when these plantations reached 10 to 20 years.

In another experiment performed in the same age sequence of plantations, Belsky, Perry, Cropper, and Belsky (in press) found that fresh needle litter contained many the initial levels of N and P, and that total needle N contents tended to remain constant and P contents to increase far over 24 months, indicating that N and P were strongly immobilized.

In these sites, with a highly weathered sandy soil and low ion exchange capacity, most of the nutrient retention and transfer are associated with the forest floor and the N horizon. It is not surprising, then, to observe positive results when N and/or P, alone or in combination with other nutrients, are added to the sites. Under non-fertilized conditions, plants may cope with poor nutrient availability by having slower growth rates to reduce requirements (Charles, 1983) or by accumulating essential nutrients in biomass components and maintaining "tight" internal cycles (Wether et al., 1984). In addition, special mechanisms or associations have evolved to enable trees to obtain otherwise unavailable nutrients (e.g., mycorrhizas for P uptake) or to inhibit certain microbial processes that may decrease losses of nutrients (e.g., nitrification and nitrinobacteria) (Pena and Belsky, 1992).

Nitrogen Mineralization and Immobilization

Mineralization is the process whereby complex organic (labile) compounds are converted by microorganisms to inorganic compounds which can be utilized by macroorganisms or released to the environment. Nitrogen mineralization can specifically be called "immobilization" as proteins, amino acids, amino sugars, etc., are degraded to ammonium-N.

Nitrogen mineralization and immobilization rates are very important parameters to consider in order to assess the N status of forest ecosystems as nitrogen availability for plants is regulated through these processes. In addition, they can be used as a measurement of the quality and quantity of organic matter substrate present in the site (Burger, 1973), and as an estimate of the proportion of the total nitrogen pool that may be utilized by the ecosystem (see Stanford and Smith, 1975).

Several interacting factors affect mineralization rates. Among the most important ones are the quantity of potentially mineralizable organic N, availability of utilizable carbon sources, C/N ratios, temperature, pH, moisture, and the presence of other potentially limiting substrates such as P.

In addition to the above factors, Anderson et al. (1981), and others, have indicated that the presence of saprophytic grasses may significantly accelerate release of N immobilized in bacteria and fungi (see also Wilson, 1971). Bacteria and fungi tend to strongly compete for ammonium-N (Winnor and Rollard, 1984), so that by reducing their population size, ammonium-N may be available to plants. In sites where such grasses are not abundant, drying and wetting

cycles may increase availability of N by reducing bacterial and fungal populations (Erick, 1980; Jurekiet, 1980; Revell, 1981).

Carbon/nitrogen ratios have been frequently used as a mineralization-immobilization index. Higher ratios will induce immobilization. Forest floors C/N ratios vary from 40 to over 80 corresponding to total N concentrations of 0.1-1.4% in an ash-free basis (Kump, 1982). For bacteria, C/N ratios range from 8-16, and are slightly higher in fungi (Meyer, 1979). As a consequence, during initial decomposition of organic matter, immobilization will prevail until C/N ratios are reduced to about 25-35.

Although C/N ratios give some idea of the rates of mineralization, other factors must be considered. Forlby (1979) found that higher mineralization rates tended to occur when labile organic matter was present, and Jansson (1979) observed an increase in mineralization when soluble organic carbon increased in the soil. On the other hand, Inspeyere (1970) concluded that N immobilization was partially related to total N content of the forest organic materials. In addition, Gundersen (1980) concluded that factors related to various grass roots inhibited mineralization, and Alexander (1977) stated that the presence of lignin in the organic matter through enzymatic processes of microorganisms which degrade proteins. These last factors may explain why pine needles tended to immobilize N (Jenny and Richards, 1977).

The examples above provide some basis for concluding that C/N ratios may poorly correlate with mineralization rates. Total carbon and nitrogen analyses do not give a complete idea of the complexity of the organic materials and do not indicate the degree of toxicity or

availability of energy sources for microorganisms present in the organic matter.

In general, increasing moisture content in field capacity and increasing temperature of the soil will also tend to accelerate mineralization (e.g., Rodeau and Vitousek, 1982). As mentioned before, wetting and drying cycles may also favor the release of N. Berling (1979) also found that low mineralization rates occurred in sandy soils when compared with clayey soils. He suggested that such a trend may be due to poor moisture retention capacity of the sandy soils which inhibits the growth of microbial populations. Finally, Gerson and Foster (1975) found a strong interaction between moisture content and C/N ratios. Mineralization was higher under waterlogged conditions only when C/N ratios were high, but no differences were observed when C/N ratios were low.

Mineralization rates may also vary with stand age and stand succession to an ecosystem. Mortenson and Flanagan (1981) noted that N mineralization in a soil from a primary forest increased initially but leveled off with time, while in soil from a secondary forest it remained relatively constant. Highest rates were observed in the oldest secondary forest. Perry (1983) and Shultz, Perry, Grogan and Hendry (in press) found no significant differences in decomposition when slash pile needles from an age sequence of plantations were studied after decomposing in the field for one year. But after 24 months, decomposition and N mineralization rates tended to decrease in the needles coming from the oldest plantations. Less, but highly significant, N immobilization was observed with an effect of stand age. On the other hand, Jones and Richards (1977) found that when

pine needles from a 20-year-old slash pine plantation were added to incubating soil, heterotrophic bacteria and fungi were stimulated to immobilize ammonium-N. Berg and Thelen (1981) determined that decomposing Scots pine (*Pinus sylvestris* L.) needles also tended to immobilize N, but only when initial N concentrations in the needles were low. Such accumulation lasted up to 2 years in when approximately 80% of the mass was lost.

Berg (1980) observed that in sites where species associated with ectomycorrhizal fungi predominate, new litter, very difficult to decompose, accumulated. Zieger and de Silva (1981) suggested that such a phenomenon may be due to the ability of mycorrhizae to absorb essential mineral nutrients from the litter, to the ability of mycorrhizae to absorb essential mineral nutrients from the litter, to the ability of mycorrhizae to rapidly utilize easily available C substrates, and/or to the ability of these fungi to produce antibiotics. All these conditions would tend to reduce N mineralization.

Forest management practices may also have an effect on mineralization rates. According to Burger (1978) and Harris (1981), mineralization rates increased with intensity of site preparation, but initially mineralizable N was significantly reduced and was almost completely depleted at the end of two years in the most intensively prepared sites (including chopping, burning, staking, and bedding). Such changes in N mineralization patterns may be related to changes in temperature, moisture content, and availability of substrates and organic carbon in the soil.

Fertilizer applications may have different effects on this process. Overholts (1967) observed that addition of nitrogen's NO_3^- -N or

NO_3^- -N stimulated N release from organic matter (a phenomenon he termed "priming effect"), but that Nite effect was observed when urea was applied. On the other hand, Rasmussen (1977) stated that addition of urea, and to a lesser extent, ammonium sulfate, stimulated release of organically bound N, mainly due to an increase in soluble organic carbon after fertilization.

Prescribed burning also has a similar stimulatory effect, as fire tends to increase nutrient contents and pH in the soil, tends to decrease C/N ratios, and maybe degrades humus stored in the forest floor (see review by Salzer, 1978). In some pine plantations in Florida, Burger (1977) found that such changes were very temporary, but were still sufficient to significantly change the N fluxes and pools of the site.

Nitrification

Nitrification, or the oxidation of ammonia to nitrate, is actually restricted to autotrophic bacteria, mainly Nitrosomonas and Nitrobacter. During this reaction, energy is released to assimilate CO_2 .

Focht and Harrison (1977) indicated that soil pH, moisture content, oxygen supply, and temperature are the most important parameters controlling nitrification when sufficient substrate is available. They found that optimum nitrification rates occur in neutral or slightly alkaline pH, with moderate moisture contents and at a temperature between 30° and 35° C.

For these factors, interest is varied, e.g., as nitrification has been observed to occur in a great range of conditions. For example, Kowalenko and Cameron (1978), during an incubation experiment on a

clay loam soil, established that a strong correlation between moisture content and temperature existed. Subraman (1987), working with some tropical soils, found no correlation between organic carbon or total N contents in the soil and nitrification rates. Instead, a strong relationship was found between N mineralized and nitrification (see also Robertson and Vitousek, 1987); when availability of ammonium-N is low, nitrification will decrease (Vasudevan, 1981, Jones and Alexander, 1982) mainly because heterotrophic bacteria are considered to be better competitors than nitrifiers for N sources (Jones and Alexander, 1982, Vasudevan, 1987).

Subraman (1982) also found that pH was only correlated with nitrification when values were below 5.0; a negative relationship was observed when acidity increased. In alkaline conditions, no significant differences were detected with changes in pH. Because of the potential effect of low pH's on nitrifiers, acid forest floors and soils are expected to have very low populations of such bacteria (Smith and Mitchell, 1984, Koway, 1986). Yet Harper (1978) indicated that for some slash and liability forests in Florida, nitrification proceeded even at pH's around 4.5, a pH lower than what is usually reported for agricultural soils. In addition, Walker and McDermott (1978), working with acid tea soils in Sri Lanka, observed that nitrification occurred at pH's as low as 4.1. These results suggest that specialized bacteria may evolve in certain environmental conditions.

Ecological succession may also influence nitrification, but with a lot of controversy exists in the literature. According to Rice and Pendley (1982), mature forests tend to produce nitrifibacteriophages that

reduce activity of nitrophiles... In this scenario, N will be conserved as nitrate if would be rapidly lost through leaching or denitrification. In addition, vegetation which tends to preferentially absorb nitrate, as appears to be the case in early successional stages, will be outcompeted.

Much controversy surrounds this hypothesis, as various investigations report opposing results. For example, Lamb (1990) found an increase in nitrification with succession in several forests in Australia. He concluded that denitrifier activity was related to N availability of the site rather than as an evolutionary strategy (see also Benner, 1982; Allen and Atchell, 1984). Robertson and Vitousek (1991) also were not able to observe nitrification increases with successional stage, and Robertson (1984) found increases in nitrification through the early successional stages of a low land ecosystem in Costa Rica indeed, with a leveling off later. He also observed that ammonium availability along the same regulated the rate of nitrification, and that in soils at all sites most of the ammonium was transformed to nitrate. Greenland (1991), on the other hand, suggested that root growth in a tropical savanna may release toxins that will suppress nitrifier activity. Other investigations may be cited, but the ones mentioned above are indicative of the debate surrounding this topic.

As with organic N mineralization, ecosystem disturbances may lead to changes in nitrification rates. Recently, Strickland and Whitham (1991), Nelson and Vitousek (1991), Vitousek et al. (1987), and others, have reviewed and discussed in detail the patterns of nitrification and nitrate availability for more than 17 different forest

types after disturbance. In a majority of the cases, nitrate accumulated in the efflu, suggesting increases in nitrification activity. Such effects were attributed to increases in oxygen availability, through increases in denitrification rates, to reduction of competition for substrate, to degradation of potential toxins, to improvement of substrate quality, either to direct changes in environmental conditions such as mixture content, pH, temperature, etc.

Nitrogen additions may favor nitrification, as was observed by Roberts-Bailey and Bellard (1978) in fertilization trials in secondary forests in the Pacific Northwest, and Boone (1978) in a 23-year-old slash pine plantation in North Florida, although in this last case the effect was not very strong. Robertson (1980) also observed a drastic stimulation of nitrification when NH_4Cl was added to some tropical forests, with the stimulus attributed to increases in available substrate, organic matter soluble carbon, and pH. Burger (1978) found an increase in nitrification after prescribed burning which may be the result of the increase of these factors after the fire.

P fertilization has also been cited as having a stimulating effect on this nitrogen process (see review by Cole and Hall, 1981), although information on the subject is relatively sparse.

Denitrification

Denitrification is defined as those redox reactions which produce nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) gases (Dowling, 1981). Usually such activity is associated with denitrifier bacteria which utilize nitrate as an electron acceptor

when oxygen concentrations are low. In addition, Blackmer and Weaver (1980) discovered that some of these gases may also be produced during autotrophic nitrification reactions.

In general, two types of denitrification processes have been distinguished: the biological and the chemical processes (Roesler, 1982). Biological denitrification has been the more studied and is considered to be the most significant contributor of N losses from ecosystems.

Environmental requirements for optimum biological denitrification rates have been extensively reviewed by Smith and Focht (1977), Roesler (1982, 1983), and Friesema (1982), although the majority deal with agricultural systems. Among the most important factors favoring denitrification are: low soil oxygen concentrations, neutral to slightly alkaline pH's, relatively high availability of C and NO_3^- , and temperatures around 20° C. In addition, denitrification tends to be higher with wetting and drying cycles, and in soils with high clay content. Plants also may exert a strong influence on this process (Friesema, 1982) by supplying various rich substrates, and by reducing oxygen concentrations in the rhizosphere. In the other hand, plants may reduce denitrification by competing for N substrates, especially in soils low in N (Smith and Focht, 1979).

Under acidic soil conditions denitrification decreases rapidly. Two major explanations have been proposed. One is that nitrate may be used in the assimilatory reduction to ammonia (Weaver and Black, 1980; Roesler 1981, 1982), decreasing availability of substrate, and the second is that nitrates, a necessary element in the enzymatic

system of denitrifiers, may become limiting under this condition (Pridmore, 1982).

Although low pH's tend to inhibit denitrification, Winkness and colleagues (1981) found that in some tropical soils, oxidation of nitrous oxide and dihalogen gases was occurring even at a pH of 4.0 when soil was amended with glucose. This suggests, as in the case of nitrification, that certain denitrifier bacteria may be adapted to special environmental conditions, although under these low pH's, chemical denitrification may be occurring (Jalun and Brenner, 1981; Jorison, 1982).

In general, forest soils have been considered to have low biological denitrification rates not only because of the generally acidic conditions but also because of the low substrate availability (Gasey, 1980). Jorison et al. (1981) found, in an extreme example, that potential denitrification in some deciduous forests may be high and may exceed stream N losses by as much as 200 times. In this study, highest rates occurred in the leaf and wood litter, but the largest total amounts occurred in the soil. Of the estimated 18.1 kg N/ha-yr denitrified, 40.35 was lost from the soil.

In a separate plantation grown on a South Florida Muck soil, Heller (1979) detected high gaseous losses of N. He determined average rates of 150 kg N/ha-yr with peaks following disturbance events. On this site, high rates would be expected because of large amounts of available C and probably also N. For other agricultural areas, N losses through this process have been estimated to be between 5 to 75%, with a mean of 25 to 30% (Pridmore, 1982). This suggests that in certain situations, losses may be considerable.

On the other hand, Reesman (1979), working with a slash pine plantation fertilized with several different sources, observed that denitrification losses accounted for only 1 to 100 of the N fertilizer applied. The percentage values obtained varied depending on the N_p/N_d ratio assumed in the calculations of his results and on the type of fertilizer and rate of application. In addition, he pointed out, as in Reesman's study, that denitrification occurred in pulses following rainstorms, and that increases in available N after fertilization, especially urea, stimulated this process.

Other management practices besides fertilization may also affect activity of denitrifiers. In plantations in Florida, harvesting, chipping, burning, etc. may increase pH, moisture content, and substrate, and N availability to soils (see Burger, 1979; Morris, 1981), all of which will tend to favor denitrification. Unfortunately no study on the possible denitrification rates in these disturbed sites is available.

Burning and Fertilization

Besides denitrification, other processes may release gaseous N products to the environment (Belmont, 1982). For example, burning may volatilize 30 to 600 of the total N found in the forest litter depending on fire temperature and N content of the organic material (see review by Burton, 1977). During site preparation, burning is used, among other techniques, to remove slash and other organic debris. According to Pettichett and Morris (1981), N volatilization losses after this operation was performed in some sites in Florida ranged from 80 to 100 kg/ha (see also, Burger, 1979, and Morris, 1981). In a tropical ecosystem, Gull et al. (1982) estimated N

volatilization losses of 400 kg/ha, or 20% of the total N put, when the site was plowed and burned. But in general, N outputs due to fires are low due to incomplete burning of organic debris, low temperatures, and low penetration of flames to litter layer (Malen, 1978).

Atmospheric volatilization from other processes may also contribute to N outputs. High rates have been observed in alkaline soils dominated by sands, especially when drastic changes in temperature and moisture occur and when N fertilizers have been applied (Belton, 1962; Sorensen, 1962). Effects of plants also must be considered, as they may decrease volatilization by decreasing availability of $\text{NH}_4\text{-N}$ in the soil, or may be sources of NH_3 as was observed by Farkner et al. (1978) in senescing leaves of an agricultural plant. Additional reduction in N gaseous losses to the atmosphere by the vegetation may occur. According to Sorensen et al. (1978) some plant categories have the ability to absorb gaseous N products, although its importance to the ecosystem is unknown.

In unfertilized forest soils, atmospheric volatilization losses are insignificant (Kenny, 1980), although in fertilized forest soils such losses may vary from less than 20 (Volk, 1972) to as much as 400 (Sorens, 1971). Russian CERN found that less than 20 of the N applied was lost through volatilization. According to Bernetti and Debeti (1980) much of the variation observed between and within investigations is related to the specific site conditions, especially pH and rate and type of fertilizer, but also to the different methodologies used and to the specific time the measurements were

lates. For example, reutilization losses are large after nitrogen mining.

Biological Nitrogen Fixation

According to Postgate (1974), biological nitrogen fixation accounts for at least 50% of the bacterial N turnover in the global N cycle. Most of the N used or stored in an ecosystem has originated through this process, as very few parent materials are rich enough in N to supply these observed amounts through weathering. Paul (1977) estimated that biological fixation usually contributes 5-10% of the nitrogen circulating between vegetation and soil components, although percentages may be higher in B-poor environments.

Nitrogen fixation has been demonstrated only for prokaryotic organisms, either bacteria or blue-green algae (Postgate, 1974). Bacterial nitrogen fixers range from obligate symbionts to free-livers, and from free-living to symbiotically associated ones. Some bacteria also have photosynthetic capabilities.

Best favorable conditions for fixation rates include large amounts of available energy sources, relatively low oxygen concentrations, moderate to high moisture contents, temperatures between 20° and 30° C, and neutral pH's. For symbiotic relationships, additional factors which affect plant photosynthate production must also be considered. In addition, availability of Mo, Fe, and P, and low levels of N, especially $\text{NH}_4\text{-N}$, favor biological fixation (for detailed discussions, see reviews by Orskov, 1981, Graham, 1981, and Ravallin et al., 1982).

Although thermodynamically the overall reduction of N_2 to $\text{NH}_4\text{-N}$ is exothermic, releasing about 5 kcal/mol (Orskov, 1981), large amounts

of energy was necessary to break the N_2 triple bond. From 25 to 28 molecules of ATP/molecule of N_2 are utilized. This represents about 4 kg of carbohydrates/kg of N fixed (Marsalis et al., 1981).

The largest contribution to biological fixation is from the bacteria of the genus Bradyrhizobium in association with leguminous species, although other associations are important, such as the one present in species like Alnus. Due to the symbiotic relationship, fixation efficiency is greater than with saprobialic organisms. For Bradyrhizobium, the efficiency rate has been calculated to be between 0.18 to 1.25 g N fixed/g of C used, while in the case of Frankia, the rate is only about 0.04-0.07 g N fixed/g C (Stewart, 1979; Fols and Sarrasin, 1988).

Average fixation rates for forest ecosystems are about 10 kg N/ha-yr (Gibson and Hardy, 1979; Cole and Rapp, 1980), varying from as little as 1-2 kg/ha-yr for free-living association, to over 100 kg/ha-yr for some symbiotic relationships.

For coniferous forests, rates of fixation have been considered to be low (Gibson et al., 1974; Jones, 1978) but amounts fixed may represent a significant portion of the vegetation N requirements. For example, Delmon (1984, 1987) estimated that Douglas-fir (Pseudotsuga menziesii Mill.) annual N requirements ranged from 17-28 kg/ha, of which 8-10 kg/ha was supplied by biological fixation. Marshall and Lindberg (1978) determined that N fixation varied from 0.3 to 28 kg/ha-yr for spruce (Picea abies L.) forest, and Scots pine (Pinus sylvestris L.) forests in Norway. Richards (1984), and Richards and Benge (1987) estimated rates as high as 50 kg N/ha-yr for an immature coniferous forest. For deciduous forests, Todd et al. (1979)

calculated rates of about 15 kg N/ha-yr, with highest values observed in leaf and wood litter components. These works suggest that generalizations are difficult due to the large variability between the sites, species, and techniques used in the measurements.

Some studies to determine the fate of the N fixed products, in the case of non-symbiotic relationships, have been performed. Richards (1971) indicated that the nitrogen fixed was rapidly absorbed by the plants, especially those associated with mycorrhizas. Jones et al. (1984) and Jones (1986) also found that losses of biologically fixed N were low when Douglas-fir and larch (*Larix laricina* S. Henry) seedlings were studied, although some denitrification occurred. They also observed that most of the products were absorbed directly by the roots, but others required to be mineralized.

Psychrophilic N fixation may also constitute an important source of N for ecosystems. According to Nelson (1981), N fixed by free-living bacteria is lower than some tropical subtropical species, but help replenish N pools of the site after it is left in fallow period. For some temperate ecosystems, Todd et al. (1978) estimated rates of only 0.15 kg N/ha-yr for an oak-brickley forest, while the soil component reached a value of 0.5 kg/ha-yr. In the other hand, Dettinger (1984, 1987), Jones (1975), and Jones et al. (1978) calculated rates of 14-20 kg/ha-yr in Douglas-fir samples. The higher rates observed in coniferous forests when compared to deciduous forests, has been attributed to the longer leaf retention which permits the establishment of the nitrogen fixers. However, other factors are involved as Brinkman and Lindberg (1979) found only low/medium rates in the coniferous forests they studied.

Wood debris and litter may be potential sites for biological N fixation, as both of them are rich in energy sources and contain low levels of N (Silva et al., 1974). Garbaye and Inchausti (1972) attributed an annual input of 0.8 kg N/ha-yr to decomposing classified (*Calluna* spp.) logs. This represented about 40% of the total N of the ecosystem. Garbaye et al. (1979) found similar rates (0.7 kg N/ha/ha-yr) in western European wood debris. Rates varied depending on decay stage, tree species wood, and type of fungi present. Piconcelli et al. (1979) determined rates as high as 14.3 g N/ha-day when they sampled the litter layer of eucalyptus forests in south-western Australia. They suggested that this result helps explain in part the N concentration increases observed over time in the litter layer. Finally, Silvester and Bennett (1972) detected significant fixation activity in forest floors of several coniferous stands in New Zealand, especially in the litter and fermentation layers.

Fixation rates also have been associated with the rhizosphere of conifers. For example, Silvester and Bennett (1972) found high activities near or within the root system of coniferous trees. They also discovered that some roots contained modified spirorbol short prolongations which apparently hosted nitrogen fixers. When they washed these roots, 50% of the activity disappeared suggesting that they are externally associated. Graham (1971) also determined that the rhizosphere of coniferous trees, such as slash pine, strongly stimulated fixation, although he did not observe any sort of root specialization. Both aerobic and anaerobic bacteria were isolated from these roots, but apparently only the anaerobes were able to

within the root nodules. Efficiency of fixation is lower in *amorphous bacteria* (2011, 1979).

Not all root environments are able to support nitrogen fixers. Jones (1978) found no strong activity in root systems of Douglas-fir trees, and Serradellera and Segarra (1977) concluded that certain *Arizobium* species produced allelochemicals that reduced fixation and prevented nodulation in legumes.

The ecological significance of biological nitrogen fixation is most evident in pioneer and early successional stages of a site. In primary series, organisms such as blue-green algae and lichens have a substantial colonization advantage over other organisms so they are relatively independent of substrate N status (Hartwigson, 1982). In secondary succession, especially in the early stages, plant species associated with nitrogen fixers tend to dominate the site as the demand for N increases through competition increases (Nelson et al., 1978, Hartwigson, 1982). Once N levels are high, or P becomes limiting, the energy costs of maintaining the symbiotic fixers may become a great disadvantage when compared with non-fixing plant species. This is evident in nature ecosystems where heterotrophic nitrogen fixers predominate over the symbiotically associated species (Gersselt, 1971). In analogous situations occurs with species associated with sporophytes (see Jones, 1980a, b); when nutrients are limiting, species associated with sporophytes dominates the ecosystem, but when nutrient availability is high or extremely low, the reverse occurs.

As expected, N-fixing efficiency decreases substantially in non-symbiotic relationships. In early successional or disturbed

loads, values of 20 kg/ha-yr or more have been recorded, while average values between 2.5 and 10 kg/ha-yr were obtained in undisturbed or active ecosystems (Hansen, 1979; Fiskerjogren, 1982). These high rates associated with the disturbed areas may be an important mechanism to reduce N stress, as much of the N pools of the ecosystem may have been lost after the disturbance (see Vitousek and Matson, 1987).

Finally, nitrogen fixers have an important role in aiding decomposition of organic matter in the soil, especially in those substrates having high C/N ratios by increasing N content of the material. Bacteria with N fixation capabilities and other heterotrophic bacteria would tend to colonize those sites as they become less selective for C sources, although in some substrates high in lignin content, fungi will tend to be present, too (Graham, 1982). After C/N ratios decrease, other types of organisms can invade such habitats (Hansen and Vitousek, 1979; Graham, 1982). Nitrogen fixers are also symbiotically associated with non-flowering plants, such as legumes and pine bark beetles, which apparently aid in the digestion of the wood ingested (Gutman, 1973; Bridges, 1981).

Management practices may affect N fixation rates. For example, because heterotrophic fixers are strongly dependent on external energy sources, vegetation and slash removal during site preparation may drastically reduce fixation rates (Graham and Linker, 1984), but may be enhanced in windrows or slash piles. On the other hand, application of N fertilizers, especially those containing nitrate, will decrease nitrogen fixation activity (Spiff and Gbure, 1972; Hargrave et al., 1982) but, the addition of other nutrients, such as P or K,

may have opposite results (Ludgren, 1978; Bradford, 1981). In addition, prescribed burning will tend to stimulate nitrogen fixation, not only because detritus is released, but also because pH tends to increase, carbon sources may become more available, and factors detrimental to nitrifier bacteria may be eliminated. Jorgensen and Wells (1981) observed higher fixation activity after a heavily pine (*Pinus taeda* L.) forest was burned; mean respired values of 20 kg N/ha-yr , although spatial variability was very high. Other examples of effects of fire on this nitrogen process have been reviewed by Nelson (1978).

Nitrogen Transport in Soils

An understanding of N movement in soils is important for two main reasons. First, significant losses of N, especially nitrate, may occur through leaching, and second, N reaches the root absorption zone primarily through mass transport.

Many studies and models dealing with N movement in soils have been performed to aid for agricultural systems. Rao et al. (1984) described a simulation model which accounts for microbial transformations, ion exchange processes, and transport of the various N biotic species. Davidson and Rao (1984) evaluated some simple models of N movement in soils which also consider physical, biological and chemical aspects. Wilson et al. (1978) also presented several models and equations to describe leaching processes in soils. Recently, Nielsen et al. (1985) reviewed this topic discussing deterministic and stochastic models, as well as problems associated with spatial variability and sampling procedures.

For steady state conditions, one-dimensional flow of B in the soil may be described by using the following equation (See, pers. comm., [SO₂ team, 1981]):

$$\frac{\partial C}{\partial t} + \frac{\partial q}{\partial x} = \frac{\partial}{\partial x} \left(D \frac{\partial C}{\partial x} \right) - \frac{\partial}{\partial x} (vC) \quad (1)$$

where

$\frac{\partial}{\partial t}$ - partial derivative

v - volumetric water content

t - time

k - bulk density

C - B concentration in the absorbed phase, $\mu\text{g/g}$ or linearly dependent on C

D - total dispersion coefficient = to the sum of effective molecular diffusion, + mechanical dispersion, + dispersion caused by diffusive solute transfers between stagnant and mobile water around soil particles

x - distance

q - Darcy's water flow

C - concentration, $\mu\text{g/g}$.

Several factors are considered to be constant through time (v , k , ρ_s , and D) and B transformation are assumed to follow Michaelis-Menten zero or first-order kinetics. The above equation (1) indicates that movement of B in the soil may be very complicated as many parameters must be studied simultaneously. Complexity will also increase if some of the assumptions do not hold, and if spatial variability, due to chemical or physical aspects, is high.

nutrient losses through leaching may depend on the N species. Nitrately ammonium is not easily leached out from soils having high cation exchange capacities. In addition, in clayey dominated soils with ill layer silicates, ammonium-N leaching may be reduced not only because of electrostatic attraction to the soil particles, but because it may be strongly fixed or trapped in the lattice structure (see review by Bockheim and Velthuis, 1982). Such ammonium-N will become less accessible for nitrification and volatilization losses (Gardner, 1982). In the case of nitrate, losses are generally high, except in acidic soils containing high quantities of amorphous materials, where absorption may be significant (Rajag and Freck, 1971).

Sandy soils have low cation exchange capacities (mainly depending on organic matter content and pH) and do not contain any amorphous materials to retain and reduce N losses through leaching. Nonetheless, Morris (1981) found that even after intensive management practices, N leaching, detected by lysimetry, was minimal in a fibrous soil. Also Rickard et al. (1984) and Rickard (1985, 1987) reached a similar conclusion when they analyzed runoff water concentrations from the same watershed. This suggests that some other mechanisms of retention may exist in this area, such as immobilization in the organic matter (see Vitousek et al., 1987), or that some of the N was lost through denitrification. Also such results may be related to the low fertility of the area so that when the sites were disturbed, availability of nutrients did not increase significantly.

In other sites, results have been different. For example, Reddy [1979] found significant increases in H^+ and other nutrient concentrations in soil solution samples after an ecological plantation in California was cleared. Also, Smith et al. [1979] detected large H^+ losses in runoff water after a Pinus forest in northeast Florida was harvested to establish a pine plantation. Levels decreased rapidly, and returned to control conditions a short period after the disturbance.

Application of nitrogen fertilizers to sandy soils will rapidly increase soil solution concentrations of this cation and as a consequence, potential leaching losses is expected to increase. Although no direct measurements were performed, Sposito [1979] suggested that an elevated portion of the applied fertilizer may have been lost through this process. He also suggested that when urea-N was applied, leaching losses may have been relatively lower because the fertilizer tended to increase pH which in turn may have helped increase the cation exchange capacity of the soil (see also Dickerson-DeLong and Gifford, 1979).

The amount of H^+ leached will vary from site to site depending, among other things, on degree of disturbance, rainfall patterns, soil type, rates of plant uptake, nutrient status of the soil, etc.,. Vitousek and Matzlik [1979] recently reviewed and discussed some of the patterns and mechanisms of H^+ losses observed in disturbed forest ecosystems.

Finally, it is important to mention that macropores and soil particle aggregation may play a significant role in H^+ movement in soils, especially in clayey soils. Under unsaturated conditions, soil

solution will move through relatively small pores inside aggregates leading to high storage of nutrients but when soil saturates, solution will rapidly move through macropores (see Bloom, 1981). In this last case, potential losses of available nutrients may be greater. In sandy soils, where aggregation is usually poor, chemical rather than physical properties, such as water repellancy, may influence water and solute movement (Bloom, 1984).

Nitrogen Uptake by Plants

Nitrogen will reach plant roots mainly by mass flow, that is, the convective flow of the solution to the roots as a result of plant transpiration (Barber, 1981). According to Barber and Bloom (1984), about 30% of the N absorbed by corn is supplied by this process, although in species associated with nitrogen fixers this proportion is decreased.

Several models for N transport to roots have been developed, mainly for agricultural conditions. One of such models was proposed by Cassman and Barber (1978), and includes 10 basic parameters: 1) describing the relationship between net plant nutrient influxes and nutrient concentrations in the soil solution, 2) describing the effect of root surface area and increases over time, 3) describing the rate of nutrient supply to roots from the soil, and the last one describing the rate of water absorption (see also Barber, 1981). Two other models frequently used in the literature are those proposed by Holt and Ransom (1970) and Blank and Banks (1970), but in general, they are relatively complex models difficult to handle in practical situations. A simpler model was described by Jordan and Lee (1971), but the

number of absorptions increases. Further discussions on solute transport to the root area are contained in Igo and Tinker (1977).

Once N reaches the rhizosphere, several factors will affect its absorption. Absorbing elements N availability, plant uptake is influenced by root system size and rate of absorption/area of root (Barber, 1981). In addition, uptake may be significantly enhanced by mycorrhizae not only because of increased surface area, but also because mycorrhizae have the capability to extract relatively unavailable N sources from the soil, with moderate absorption rates, and can effectively compete with other microorganisms for N substrates (Jensen and Smith, 1981). Also Kuhnemann et al. (2001) discovered that mycorrhizae of secondary vegetation may be directly interconnected with nitrogen fixing plants in the understorey. By such connection, the authors suggested that N nutrition of the secondary vegetation may be significantly improved.

Transport of N from the root to the shoot will not be discussed here. Girty (1971) reviewed this subject as well as the assimilation and excretion of the different N species by the plant, and the effect of N on growth.

In mature temperate forests, ammonium-N tends to predominate in soil solution (Vlassak, 1976; Ray et al., 1979; Ransap, 1980; Gals, 1980) suggesting that forest vegetation may obtain N mainly as ammonium-N. Hies and Fiedholz (1975) claimed that the absorption of such N species is very advantageous as it may be transported directly as amino acids. In contrast, if nitrate-N is absorbed, this must be transformed with the excretion of high amounts of energy. But its predominance in the soil solution may not provide sufficient

justification for concluding that ammonium-N is the preferentially absorbed N species. For example, Patterson (1982) indicated that in most tree species, ammonium-N uptake occurs best in neutral pH, but absorption decreases rapidly in acidic conditions, such as those found in many forest sites. This suggests that maybe the abundance observed is related to lower absolute uptake rates. Other interesting considerations in relation with this topic, were presented by Patterson.

In the other hand, there is some evidence that the form of N absorbed by plants may vary with successional stage. Barnes (1977) observed a shift from nitrate to ammonium-N uptake in an old field to a Pinus-Sargassum successional zone in South Carolina. Queller (1981) also suggested that early successional species in a tropical wetland tend to absorb only nitrate-N. In his experiments with Lythrum floridanum, a species characteristic of mature ecosystems, and Salvia mexicana, a pioneer species, he showed that Lythrum spp. grew better in soils containing ammonium-N, while the contrary occurred with the pioneer species. These results must be considered in fertilization trials, as type of fertilizer used may stimulate growth of different species.

In addition to N uptake through roots, some investigators, such as Jones (1980) and Freney et al. (1981), have indicated that some species, including cereals, may obtain N through leaves. And the significance of this process in relation to the general N requirement of the plant is not well known.

Nutrogen requirements vary depending on site fertility, growth stage, species, and other factors. Ingstedt (1978) suggested that N

status of seedlings may strongly affect and may be used to predict N requirements of the plant in the future.

According to the review by Cole and Bell (1981), coniferous deciduous forest requirements for N ranged from 34 to 84 kg/ha-yr, while deciduous forests, on the average, need more N. This may be partly related to greater leaf retention by the conifers, slower growth rates, and higher internal recycling. For deciduous forests, Cole and Bell estimated that for each kilogram of N absorbed, about 140 kg/ha of above-ground biomass was produced annually.

N-P Interactions

Little information exists on the possible interaction between N and P cycles, although some concepts were reviewed by Cole and Bell (1981).

N and P cycles are expected to be closely associated because of the high energy requirements in N transformations which must be met by the presence of ATP. Cole and Bell (1981) hypothesized that N reaction rates will eventually equilibrate with P availability, that is, P will become limiting for such N processes to proceed.

General N processes are known to be affected by P levels in the soil. Janda (1980a,b) and others have indicated that the increase in nitrification and biological nitrogen fixation in these legumes associated with nitrification may be related to the ability of symbiotic fungi to supply the plant with adequate amounts of P (see Cole and Bell, 1981). However and Wolfson (1977) found that additions of P increased the amount of N absorbed, and concluded that available P was the rate-limiting factor of organic N release from organic matter. Forchum (1974) determined that low P levels affect nitrification

rates, and concluded that nitrate oxidizers were more sensitive to P availability than ammonium oxidizers.

Although experimental field results are scarce and hard to interpret, there is convincing evidence that suggests strong P-K interactions. Studies including both K and P availability levels will be necessary to more fully understand & cycling processes of ecosystems, adding another dimension to the already complex analysis.

Variability in Field Sampling of Soil & Aquifers

Spatial and temporal sampling variability can seriously affect the accuracy and precision of field measurements, which may lead to erroneous interpretation of results obtained. Nevertheless, little attention is generally given to these sampling problems solely because it would inevitably require longer and more expensive experimentation.

As summarized by Chapin (1984), assessment of the degree of spatial and temporal variability of a process to be investigated, will depend on the number and size of samples taken, nature and type of components measured, environmental conditions of the site at the time of sample collection, sampling method, and techniques used in the chemical and physical analyses of the samples. In addition, history of the site may be required to determine possible sources of variability.

Spatial variability can occur at micro- and macro-scale levels. Studies which have very small sites, such as in microbiological investigations, generally contain high variation. For example, inside aggregates in clayey soils denitrification may be occurring, but outside the aggregates there may be sufficient aeration to inhibit this process (Greenland, 1976). On the other hand, the larger the

with sample size the higher the expected variability or the probability of including heterogeneities increases. In both cases, prohibitively large numbers of samples may be necessary to satisfy all statistical and experimental objectives.

Temporal variability must also be considered. Many processes in nature occur in bursts or pulses. Several physical, chemical and biological parameters must interact together to produce a determined effect that will trigger a specific process. For example, Morris (1981) performed his experiments in a dry year and concluded that, in those conditions, nutrient losses were low. But such results may not be applicable in a wetter year.

Another example of the possible problems associated with ignoring both spatial and temporal variability, was discussed by Westerman et al. (1981) for 8 cycling investigations in grasslands. They pointed out that only research studies have assumed that the site is homogeneous, without testing homogeneity, but the analyses performed in small areas were extrapolated to the whole site. Another common mistake was to assume that processes did not vary with time, while the data collected were used to calculate annual budgets and fluxes. In one of the studies by Westerman et al., for example, they observed that during one specific year a nitrogen fluxation rate of 64 kg N/ha was estimated, but in the two subsequent years, only a rate of 2 kg/ha/yr or less was found, a difference of at least 30 times from one year to the next.

It also is important to distinguish between natural variation produced by environmental conditions, and those produced artificially as a result of the method used. This point may be exemplified by

using Hassan and Harris's [1978] work, where a substantial bias and variability in nitrate concentrations were found in samples collected from porous cup samplers. Variability was caused by different water levels of the cups, by differential plugging of the pores of the samplers over time, and by the various positions in which the samplers were placed.

Recently, a number of workers have discussed the variability problem in sampling soils and vegetation. Bigger [1984] reviewed the causes and patterns of spatial variability in soil N processes, and Harris and Nielsen [1988] dealt with the variation in other physical soil factors. Chapuis [1981], Lee et al. [1984], Inley et al. [1981], and others, have studied the variability and distribution patterns associated with nutrients in vegetation. It is important to mention that these and other authors have found that their data did not follow the requirements of normal parametric statistics.

In conclusion, many factors must be considered in order to properly interpret experimental and observational data, although as mentioned before, technical or economical support and availability of time may not be adequate.

Methodological Considerations

In the following sections, a brief review of the several methods used for measuring some of the N processes to be used in this investigation will be presented. The purpose of this section is to provide some justification for the selection of some of the methods used in this investigation.

Nitrogen-fixation-potential and Identification Techniques

Several procedures have been proposed to measure the actual or potential ability of microbes to transform and release N that may be later utilized by plants. Recent reviews on the subject include those by Nishio and Nannip (1975), Nannip (1980), and Broadbent (1981). These procedures have been divided into two broad groups, chemical and biological.

In the chemical methods, soils are extracted with different compounds and analyzed for N in the extracts. These methods are considered to be more precise and rapid than the biological methods (Broadbent, 1981), and according to Nannip and Bremer (1984), they are less affected by soil pre-treatments. The major criticisms are related to their inability to simulate microbial activity, and, as a consequence, rates of N transformation and release over time under field or laboratory conditions cannot be estimated (Broadbent, 1981).

Biological procedures include both *in-situ* and *in-vitro* incubations. In these cases, soil samples are incubated in the field or laboratory for a period of time and changes in N concentrations (both ammonia and nitrite-nitrate) are determined. Starmer and Smith (1970) proposed a laboratory method to estimate the N mineralization potential and mineralization rate constant for a specific soils type. Basically, the method consists of incubating a pre-leached soil sample under constant moisture for 30 weeks at 20° C, and extracting at several times during the incubation period with sodium chloride and a 5-min solution. A maximum cumulative N concentration level is reached which apparently correlates with actual mineralization rates. According to this and later studies (e.g.,

Harford et al., 1994), a first-order kinetics model can be used to evaluate these nitrification potentials, although recent investigations by Smith et al. (1992) suggested that such model may give errors and that other models should be used.

One of these tests have also been strongly criticized because they involve severe manipulations of the soil, such as stirring and pre-leaching treatments, and because they do not take into consideration plant uptake of N and do not simulate field variability (Gasey, 1989; Broadbent, 1991). In addition, soils aerobically incubated, more than those anaerobically incubated, are significantly affected by air drying prior to incubation, by substrate correction during the incubation period, and by the initial status of the soil (Gasey and Freeman, 1994).

One of the soil often used methods to directly evaluate nitrification rates under field conditions was proposed by Lee (1966)--- in this case, a soil core is extracted, covered with polyethylene plastic to reduce water losses, and left in the field for a period of time, usually 30 days--- Yet, according to Freeman and Douglas (1991), most commonly used plastic films are unable to tightly restrict O_2 or H_2O , and permeability to such gases varies depending on temperature and moisture content of the sample, affecting the rates of the aerobically mediated N processes.

Some advantages of the biological incubation methods include their ability to better simulate microbiological conditions (Broadbent, 1991), and the fact that both nitrification and, in the case of the aerobic incubated soil samples, nitrification processes may be measured simultaneously (Vitousek et al., 1987). Powers (1960)

and others indicated that the ammonia fixation results correlated well with tree growth (see also Isenby, 1980).

Protein-Nitrogen Fixation

Isotopic techniques have been used to measure N_2 fixation activity. These have been extensively reviewed by Barlett (1984), Isenby and Salzman (1987), Boersman (1988), and Kuntze et al. (1989). Procedures can be grouped as direct or indirect methods.

The direct methods measure N_2 fixation without requiring conversion factors. One approach used is to determine total N changes with time in the soil or in the nitrogen fixation plant. But, because of its low sensitivity, large intervals of time and systems with high fixation activities are needed. In addition, if an open system is used, other fluid movements must be made to include other possible inputs and outputs. Another approach is to use $^{15}N_2$ isotopes. This technique is about 100 times more sensitive than measuring total N changes with, for example, the Kjeldahl procedure, but is very expensive, requires sophisticated instrumentation, and is time consuming. Also, complications apparently increase when studies are performed in the field, causing problems in interpretation.

Several indirect methods are available. The acetylene reduction method is one of the most popular ones. Among its most notable advantages are the following:

- 1) It is relatively inexpensive and equipment is readily available;
- 2) It is very sensitive;
- 3) Relatively undisturbed samples may be studied;
- 4) The method can be adapted to field studies without major modifications.

Some cautions:

- 3) Because of its sensitivity, the method can be used in systems with low fixation rates.

But, the acetylene reduction method also has limitations. These have been discussed by Jitty (1979), Howden (1981), Turner and Howden (1980), and Howden and Howden (1981). Among them are the following:

- 1) Because method is very sensitive, very careful handling of samples is required. Changes in microbiological activity produced by soil variations in temperature, moisture, pressure, or aeration during sample handling may be induced and, as a result, variability of data may increase. Short incubations are recommended for this reason.
- 2) Besides nitrogen fixation, other microbial processes such as ethylene oxidation are affected by the acetylene gas.
- 3) Controls used to determine ethylene production in samples with no acetylene, are not adequate. Ethylene usually is oxidized very rapidly by microorganisms, but in the samples containing acetylene gas, such oxidation is inhibited. As a consequence, N fixation rates are overestimated. This problem is usually of greater significance in soil systems having low fixation rates.
- 4) A conversion factor is required to transform ethylene produced to actual nitrogen fixed. This factor varies depending on the sample used.
- 5) Diffusion of acetylene and ethylene between sampling ports and fixation sites, may cause apparent loss and non-Linear results.

Despite from these disadvantages, the acetylene reduction technique is still widely used due to its simplicity, sensitivity and relative cheapness.

INTRODUCTION

Geographical Location of the Study Area

The study area of the present investigation is located in Bradford Co., Pa. (lat. 40° 52' N, long. 80° 05' E), an property owned by Continental Corporation of America and managed for commercial production of poly. Ester by steam plate (Fig. 1).

Geology and characteristics of the study used here were described by Sholz and Fisher (1982). In general, terrain at all plots is flat and soils are poorly drained with high levels with a subsurface organic horizon (O_{sh}) within 60 cm of the surface, and basal ferric clay layer (A_{sh}) around 300 cm. These study soils have a low ion exchange capacity (3-5 meq/100 g) with the majority of the exchange sites associated with organic matter (Turn et al., 1987; Pritchett and Cameron, 1981). They are highly weathered, acidic, and very infertile with low organic matter contents (Sholz and Fisher, 1982; Sholz and Fisher, in press). Organic matter and nutrient dynamics in an age sequence of plantation stands, including the 6- and 28-year-old plots used here, have been described by Sholz and Fisher (1982), Sholz, Perry, Cropper, and Dewey (in press), and Sholz, Fisher, and Pritchett (in press).

The climate of the region is warm and humid with a mean annual air temperature of 26.0° C and a minimum of 6.0° C (January), a mean annual precipitation of 1120 mm, and a mean soil temperature of 18.0° C. Some specific conditions encountered during the experimentation



Figure 2. Map of Florida and an inset showing the location the plots used in the present investigation.

plots, including water table levels, are presented in Fig. 3 and Table 4.

Characteristics of Study Plots

Three 110 x 55 m plots were selected in a recent clearcut site, and in a 5- and 25-year-old plantation. Within each plot, 11-d m diameter circular subplots, each separated by 20 m, were established. Prior to the recent harvest, all plots had not been fertilized, thinned, or harvested for at least 8 years (Mois and Fisher, 1982). All sites present comparable soil conditions, and site preparation in all consisted of roller chopping with a weighted drum, burning, and machine bedding and planting.

In general, the vegetation of these plots may be described in several ways. Among the most important factors are the very low availability of nutrients in the soil, especially P (Pritchett and Goodford, 1983; Mois and Fisher, in press; Mois, Fisher, and Pritchett, in press), and the extreme changes in water regime, especially near the surface where greatest abundance of fine roots develop (Mois and Pritchett, 1984). Due to the proximity of the water table to the surface, sites flood easily (Fig. 3) during rainstorms, but surface water drops very fast in the dry period.

Clearcut Area (20 Apr)

The clearcut site (8 up), which was harvested in March 1983, had been part of the same plantation block that contained the 25-year-old study plots. Trees were cut at the 110-m surface, branches removed and left in the area, and stems harvested. No further operations were made. Minimal disturbance of the mineral soil occurred, although, because of some displacement in the litter layer, several portions

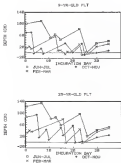


Figure 3. Water table levels reported during the three experimental phases to the 5- and 25-yr-old plantations.

Table 2. Average soil temperature ($^{\circ}\text{C}$) at 5 cm, and cumulative rainfall (mm) during the three investigation phases: 16 June-July 1983, October-November 1983, and February-March 1984.

		PLANTING AGE (yrs)					
		Oldest		5-yr-old		16-yr-old	
		TEMP	RF	TEMP	RF	TEMP	RF
JUN-JUL	5	17.0 ^a	---	16.1	258	15.4	268
	20	4.8	---	1.6	---	5.3	---
OCT-NOV	5	---	---	16.4	265	17.4	264
	20	---	---	2.8	---	2.2	---
FEB-MAR	5	14.8	---	16.6	214	17.2	308
	20	1.7	---	1.3	---	1.3	---

(^a) Reported in June-July 1983.

were exposed by direct sunlight and rain drops. In addition to the heterogeneity of the litter layer, small piles of branches and small stems occurred in several places.

No aboveground live vegetation was present in the area for several months following the harvest operations, but after one year some grasses such as *Andropogon* sp. and *Panicum* sp., and saw palmetto (*Serenoa repens* (L.) Swall) plants became established in the area.

Nine-Year-Old Plantation

This plantation was characterized by having a relatively open canopy due to its young age and relatively wide spacing. This openness permitted a strong dominance of grasses in the understorey, such as those found in the disturbed site, mixed with saw palmetto, gallberry (*Vaccinium glabrum* (L.) Chap), and *Smilax* sp.

This site was prepared in 1974. Beds are still clearly evident, covering some 70% of the surface, with the balance in disturbed troughs. There was no or only a very thin litter layer present, with no holes.

Although all sites flooded at some time, this stand tended to be more prone to this condition (Fig. 3), drainage was slower than in the other areas.

Twenty-Nine-Year-Old Plantation

This plantation was a well-stocked stand with relatively sparse understorey, mainly composed of saw palmetto, and had a thick, well developed forest floor. No signs of beds or interbeds were detected, although some old fire scars were observed. A nearly stand with these same characteristics, was the one harvested and used as the clearest site.

Treatment Application

The treatments were randomly applied to 6 of the 18 subplots within each of the plots. No fertilization and prescribed burning. The remaining subplots were maintained as controls. Calcium superphosphate and potassium chloride were applied with a hand-held spreader, twice (in March and October 1981) at a rate of 50 kg N/ha and 50 kg K/ha.

Burning was done in March 1982, the same week the fertilizer was applied, on 10 x 10 m squares enclosing each of the subplots. Each square was isolated by about 2 m wide strips, marked orange paint to suppress bare the mineral soil to keep the fire from spreading. At the clearest plot, five fires were started along all inside edges of the square and allowed to burn inward toward the subplot. At the two older sites, a single fire front was established with the fire fronting upwind the wind over the subplot (i.e., backfire technique). In general, the burns were relatively hot and uniform except in the clearest plot where the litter layer was not continuous.

In addition to the above treatments, and as part of an on-going project directed by Dr. R. Gaul (School of Forest Resources and Conservation, University of Florida), a 10 x 10 m plot was established in the 10-year-old study area, about 200 m away from the other treatments. This plot was fertilized with urea, calcium superphosphate and potassium chloride annually for three years (August 1981, 1982, and 1983) at rate of 150 N, 50 P, and 50 K kg/ha. Although itself unfertilized, the plot was large enough to contain 5 subplots as described above.

Processes in the A Cycle Studied

Several microbiologically mediated N processes were studied in each subplot, including the following: net nitrification-denitrification, nitrification, and potential non-symbiotic biological N_2 fixation. In addition, variations in P concentrations and water soluble organic carbon (DOC) were investigated simultaneously with the above studies to determine if there was some relationship with the N processes.

Results from this investigation will be interpreted in the context of results at the stand-level already conducted in the series, and in this way, the subplot experiments will be integrated with other larger scale experiments.

Details of the procedures used in the study are described in the following sections. Field and laboratory experiments were conducted during three periods in 1983-1984: June-July 1983, October-November 1983, and February-March, 1984.

Measurement of Nitrogen Mineralization and Nitrification

Soil Core Extraction

Three undisturbed cores per subplot were extracted from the A horizon with a 7.6 cm diam. x 8 cm long sharpened PVC tube. Two of the sets of cores were immediately placed in wet/dry proof bags and returned to the laboratory. The remaining set was left in the field.

The cores brought to the laboratory were incubated for 30 days at 20° C. Cores were divided into two groups. In one of the groups (designated as "watered"), deionized water was added from below until the water level was about 2 cm above the soil surface. The second group ("unwatered") was maintained at its initial moisture content.

during the incubation by periodically adding dechlorinated water to keep same total core weight). The cores were open to air circulation, but to avoid very fast drops in moisture a thin plastic layer with small holes was placed on top. These two extremes of moisture control were selected to illustrate possible mineralization rates under these moisture conditions which frequently occur in the field, and to have some indication of possible mineralization rates under constant temperature and moisture (in the case of the unsaturated cores). Procedures used above were similar to those described in Gentry (1982).

The third set of cores was incubated in the field for 30 days. A novel incubation procedure was used (Fig. 4). Instead of enclosing the core in polyethylene bag, as suggested by Liu (1980), two nylon mesh bags (with a rigid ring inside that fit tightly against the inner wall of the core), each containing about 20 g (dry weight) of a mineral ion exchange resin (D18, Table 2), were inserted at the top and bottom of the core to isolate the soil. The edges of the core against the ring were sealed with silicon glue to avoid boundary flow of soil solution. In addition, a protective wire mesh screen was placed over both ends. The core was then reintroduced into the hole, making sure there was no space left between the bottom end of the core and the soil, and the bag covered with litter.

The top resin bag was designed to trap flowing ions during the incubation period, the bottom resin bag was used to trap ions leaving the soil core. In this way, a mass balance for N could be constructed to determine total mineralization and nitrification (N extracted free

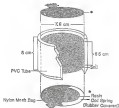


Figure 4. Diagram showing the field incubated soil core. Both ends of the core were isolated with the top exchange mesh bag.

Table 5. Chemical and physical characteristics of the mixed bed ion exchange resin (Rohm 1-300, certified grade, Fisher Scientific, Inc.) used in the B mineralization studies.

BEAD SIZE (wet, U.S. standard sieve)	20-60
TOTAL EXCHANGE CAPACITY (dry basis)	1.88 meq/g
MOISTURE CONTENT	54.3%
ACTIVE ANION GROUP	RCS_2^-
ACTIVE CATION GROUP	R_2N^+
IONIC FORM	H^+ (5%)
ACTIVE pH RANGE	2-14

the well = 8 extracted from the bottom resin bag). Other details are explained in Appendix 3.

Some potential advantages over the plastic bag technique (Tan, 1980) for which I used the resin procedure, include the following:

- 1) Diffusion of gases should not be restricted.
- 2) Mixture contents of the core can change during the incubation period.
- 3) Movement of ions by mass flow inside the well core is possible, avoiding artificial accumulation of inorganic N in the well solution. In a way, ions trapped by the low IRN ionic absorption of nutrients by roots.
- 4) Ions ammonia and nitrate are trapped by the IRN, apparently to further transformations occur (Johanson, 1983; Hinkley, in press). This reduces the possibility of N losses through denitrification, and ensures that ammonia- and nitrate-N are accurately assessed.
- 5) An estimation of potential nutrient leaching may be obtained through analysis of the lower resin bags, assuming no uptake by vegetation (as in the recent clearest study).

In addition, a recent study indicates that little ammonia- or nitrate-N reaches the roots by diffusion from the surrounding soil (Hinkley, in press), so that all the N on the roots should be a result of water flow vertically down through the cores.

To represent initial conditions of the well cores (before incubation), three replicate wells (unpore/waterbag) were collected at the same depth and without including the inner layer around the mineralisation core area. Each of the three replicates were

aggregated, dried and analyzed for ammonia, nitrate, inorganic P, pH, total nitrogen (TN), and other cations (Appendix J).

Chemical Analysis Procedures

Analysis of soil samples were performed as follows:

For the determination of inorganic P, fresh soil was stirred and screened with a 2 mm opening stainless steel sieve. In the case of the disturbed cores, soil was first stirred for 30 to 30 min before sieving [disturbed water was collected and analyzed for P, see Appendix I]. A portion of the fresh soil equivalent to 10 g of dry soil was stirred with 200 ml of distilled water for 1 hr, filtered with Whatman #42 filter paper and stored in the refrigerator at 3° C until it was analyzed (Kump and Nelson, 1982). Ammonia- and nitrite-nitrate-N concentrations were detected using the automatic colorimetric procedures of a Technicon Autoanalyzer II (Technicon Industrial Systems, USA).

Another portion of the stirred fresh soil equivalent to 10 g dry weight, was stirred with 100 ml of distilled water for 1 hr in a glass flask and then filtered with a 0.45 micropore filter. The filtrate was analyzed and digested immediately after, following the procedure by Bouas and Brown (1984), and stored for a later determination of water-soluble organic carbon (DOC). Each sample contained 1 ml of filtrate, 0.2 ml of 10% phosphoric acid, and 1 ml of a saturated solution of potassium persulfate. Samples were purged of inorganic C by bubbling with oxygen gas, and then sealed. Once sealed, samples were digested in autoclave for 30 min at 120° C and analyzed for CO₂ using an infrared (IR) detector.

Inorganic solvent contents of the fresh soil were determined by weighing 10 g of the mixed soil, oven-drying it at 100° C for 24 hrs, and reweighing.

Analysing soil was air dried for 2-3 weeks, stored, and sent for pH, organic matter, cation and inorganic P analyses to the Analytical Research Laboratory, University of Florida. This laboratory uses the following procedure to determine extractable Al, Ca, Fe, S, Mg, K, P and Na. 1.0 g of mixed soil is extracted for 5 min with 20 ml of Retic 1 extracting solution (0.08 N HCl in 0.005 N H_2SO_4 , Retic, 1981). The filtrate is analyzed by atomic absorption spectrophotometry, using a Perkin-Elmer 5000 spectrophotometer, except for P. P is determined colorimetrically using the Technicon Autoanalyzer II (Technicon Industrial Systems, 1970). Soil pH is determined in a 1:1 soil:water suspension, and organic matter is determined using the Walkley-Black test of titration method (Jensen, 1961). In addition, total nitrogen was analyzed using the micro-Kjeldahl procedure (Bremner, 1964).

Inorganic N and P absorbed to the ILE were extracted by adding 2 g of air dried resin to 50 ml 2 N HCl solution for 1 hr, and filtering it with Whatman #40 filter paper. The filtrate was analyzed for ammonia, nitrate (plus nitrite), and inorganic P using the Technicon Autoanalyzer II.

It was found that, during the analysis for ammonia extracted from the resin, some kind of contamination inhibited the color development in the Technicon Autoanalyzer or lead to overestimated when the steam distillation was used. After several laboratory tests, it was found that

- D) By increasing the concentration of sodium hypochlorite from 1 to 2 mL/100 mL, and decreasing the concentration of sodium nitroprusside from 0.20 to 0.10 g/100 mL in the technique procedure, and by diluting the sample, low levels of ammonia could be readily detected.
- E) When the above changes were made and the sample diluted 2.5 times, a linear relationship between ammonia detected and added to the TCI was obtained in the range of 0 to 5 mg/L.
- F) When a separate sodium-only resin was purchased, and the experiment repeated, no interference occurred during the steam distillation, and only a slight positive overestimation occurred in the technique. This results may indicate that the urea portion is causing the problem observed in the analysis of the mixed resin bed. A similar conclusion was reached by Hart and Bradley (1984).

In the present study, ammonia extracted from the resin was analyzed using the modifications mentioned. A standard curve relating the amount of ammonia extracted from the resin and detected in the technique, after the solution was diluted 2.5 times, to that actually added to the resin, was developed in the laboratory and used to estimate the amount of ammonia trapped in the field resin (Appendix A).

Examination of Non-Synthetic Polypropylene Resin

Unfortified soil cores (10-cm diameter x 15.5-cm long), not including the filter paper, were extracted from each subplot near the field nitrogenization cores between 8 and 10 A.M. on each of the sampling dates.

Each of the cores was sealed with a septum and a rubber "20" cap at the top and bottom ends, respectively, transported to the laboratory, and then prepared for the acetylene gas injection. Sixty ml of air were then extracted from each core, and replaced with the same amount of acetylene gas, this gave a final acetylene concentration of approximately 30% (v/v). Cores were then incubated for 8 hrs at temperatures similar to those encountered in the field during the collection period (i.e., 10° C, 18° C and 22° C for the summer, fall, and spring phases, respectively). After incubation, 5.05 ml of the soil core atmosphere was extracted with a syringe and injected into a Series Gas Chromatograph (Model 2700) for acetylene and ethylene detection. The gas chromatograph characteristics were as follows:

Oven temperature: 100° C

Injection port temperature: 140° C

H_2 flame ionization detector temperature: 150° C

Carrier gas: H_2

Column pressure: 1.41 kg/cm²

Column type: Poropak Q₂ mesh size: 80/100

Column size: 150 cm long x 0.3 cm diam.

Acetylene gas was used as an internal standard. The concentrations of the acetylene and ethylene standard gases were calculated with the standard gas law assuming 25° C temperature and 1 atmosphere of pressure.

The formula described by Hardy and Salzman (1977) was used to calculate ratios of ethylene/carbon produced and H_2 fluxes/dry. A 0.3 ethylene/ H_2 ratio, and a 35 hrs/day activity was assumed for

estimate the nitrogen fixed. Since the validity of these assumptions was not verified, and, because this experiment used short term incubations, values only represent potential N_2 fixed in the field (see Smith et al., 1985).

After the incubation, each core soil sample was sliced, sliced and air dried. Analyses for nitrate, pH, organic matter, and total nitrogen were accomplished as above (Appendix B). Biotinergic molecular constants were also determined.

Statistical Analysis

The B and P mineralization data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS). A split-split plot in time model was utilized to determine significant main effects or interactions between plantation age, treatment, incubation method, and season (Grewal and Littell, 1981; R. Lynch, pers. comm., 1984). Plantation age effects, treatment effects, and their interactions were tested with the "replication (plantation age) treatment" factor as the error term. A similar design was used for the non-cyanotic N_2 fixation, except that incubation method was not included in the model and arithmetical values were transformed to base 10 logarithms before the statistical analysis. The transformation was performed to stabilize the variances so that standard parametric statistical analyses could be utilized (Sokal and Rohlf, 1984). Final presentation of the means was obtained by retransforming the logarithm values.

A least-square means procedure was used to determine significant differences between means. Only pre-planned comparisons were used to ensure the validity of tests and obtained probability levels.

Comparisons among plantation ages, treatments, incubation periods, and seasons were made. In addition, a stepwise linear regression analysis was performed to determine if selected surface soil chemical parameters affected the B processes.

RESULTS

Ammonification

Isotermal Effects

Summer. During the summer a highly significant plantation age-incubation method interaction was found for all treatments ($p < 0.001$). Initially the 8-year-old plantation had a 30% lower ammonium-N concentration than the clearcut ($p = 0.001$) and the 20-year-old ($p = 0.001$) plantation (Fig. 5). But, after the one month field and laboratory incubation period, all areas tended to have similar levels of ammonium-N, although they were somewhat lower in the 20-year-old plantation (significantly different from the other sites only for the saturated laboratory cores).

The clearcut and 8-year-old plots had significantly higher net ammonification rates than the older plantation for all the incubation methods (Fig. 6). In the older plantation, it tended to be immobilized.

When incubation methods were compared, the field and saturated laboratory cores from the clearcut and 8-year-old plantation had higher rates of net ammonification (at least 4 mg N/kg-soil compared with less than 2) than the unsaturated cores. Rates of ammonification in the unsaturated laboratory cores were low and were significantly different from the field and saturated laboratory cores in the clearcut plot, and the saturated cores in the 8-year-old plantation.

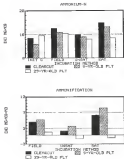


Figure 5. Summer soil ammonium concentration and ammonification rates for the various plantation ages and incubation methods

No significant differences among methods were observed for the 20-year-old plot.

In addition, a significant ($p = 0.00$) interaction between plantation age and treatment for all incubation methods was observed. In the oldest site, assimilation concentrations of control plots were not significantly different ($p = 0.05$) from the burned or PE fertilized plots (Fig. 6). In the 9-year-old plantation, control plots were significantly higher ($p = 0.00$) than burned plots, and in the 20-year-old plantation no differences among the treatments were observed. In general, net assimilation rates tended to be lowest in the burned plots, with relatively high net assimilation rates in the oldest plantation (Fig. 6).

Fig. 7. In the fall, only a significant treatment-incubation method interaction ($p = 0.05$) was found (Fig. 7). No effect of plantation age was detected. In this season, the laboratory incubation cores from the burned and PE sites showed higher concentrations of ammonium-N than controls (Fig. 7), although only the cores from the fertilized plots were significantly different ($p = 0.00$). There was also an apparent stimulation of ammonification rates by the burned and PE treatment in the laboratory cores (Fig. 7). A maximum average rate of 32.1 μg of $\text{NH}_4\text{-N}$ was obtained for the subsieved laboratory cores from the PE treatment. In the other hand, some immobilization of ammonium-N was observed in the field cores, contrary to what was found in the summer.

When incubation methods were compared within treatments, in all cases the laboratory cores had at least double the amount of ammonium-N over the initial conditions ($p = 0.00$, Fig. 7), and had the

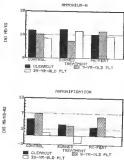


Figure 4. Sower soil amaranth-8 concentrations and appropriation rates for the various plant ages and treatments.

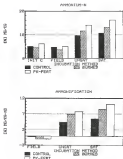


Figure 7 Fall soil appropriation control/peat and peat/peat ratios for the various incubation methods and treatments

highest net assimilation rates (Fig. 1). The field and initial conditions were statistically similar, so that no net assimilation occurred.

Spring. In the spring, a significant plantation age-isolation method interaction was again observed ($p = 0.0002$), while treatment effects were not statistically significant. As in the fall initial condition and field core values were very similar when isolation methods were compared (Fig. 3). Also, no differences were detected when ammonia-N concentrations in these core sets were compared among the plantation ages. Net assimilation rates were relatively low in the field cores for all the plantations (Fig. 4).

On the other hand, in the laboratory isolated cores, some significant differences were found. This time, average ammonia-N concentrations in the unisolated laboratory cores from the 25-year-old plantation, were approximately double the concentrations of the 5-year-old or clearcut areas ($p = 0.05$). When the isolated laboratory cores were analyzed, the 5-year-old plantation had around 100 higher concentrations than the clearcut or 25-year-old sites, although differences were not statistically significant (Fig. 5).

In general, the laboratory isolated cores had ammonia-N levels at least 100 greater than the initial conditions (significance at $p = 0.05$). The only exception was the unisolated cores in the clearcut site (Fig. 5).

Unisolated and defoliated cores tended to have higher assimilation rates (at least 4 $\mu\text{g/gms}$), except for the unisolated cores in the clearcut area. Field isolated cores had significantly ($p = 0.05$) lower (Fig. 6) field concentrations than the

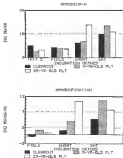


Figure 4. Spring soil amphoteric concentrations and amphoteric values for the various plant ages and incubation methods.

laboratory incubated cores for all sites and no significant immobilization was observed over the 30-day period.

Plantation Age Effects

Clearcut: A significant interaction ($p = 0.0001$) between season and incubation method was observed for each of the plantation ages. In the clearcut site, lower ammonium-N concentrations were found in the initial condition and field cores during the fall and spring seasons (Fig. 8). This decrease was significant at the 0.05 probability level, and, in some cases, amounts were almost 3 times less than the values in the summer. Fall and spring levels were statistically similar. On the other hand, the laboratory incubated cores had a significant ($p = 0.001$) reduction in their ammonium-N concentrations in the spring only. Summer values were at least double of those found in the other seasons (Fig. 8).

When seasonal net immobilization rates were compared (Fig. 9), a relatively strong drop in activity was observed in the field cores during the fall and spring seasons: from more than 4 mg N/kg-so in the summer, to less than 1 mg in the other seasons. Laboratory incubated cores showed no significant differences in net immobilization rates with season, but the incubated cores had significantly ($p = 0.01$) higher rate in the fall when compared with the summer or spring values (Fig. 9).

Five-year-old plantation: In the 5-year-old plantation, field ammonium-N concentrations also tended to decrease in the fall and spring (Fig. 10). Many similarities were observed when this plantation age was compared with the clearcut area. In the 5-year-old site, initial concentrations were half the concentrations of the

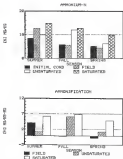


Figure 8. Soil arsenic-15 concentrations and amplification rates in the plover site for the various seasons and location effects.

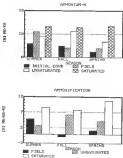


Figure 10: Soil ammonium-N concentrations and ammonification rates for 204 9-year-old plantations for the various seasons and incubation methods.

spring ($p = 0.00$) although they were similar to those of the fall. Field microbial cores also had significantly lower ($p = 0.00$) concentrations in both the fall and spring seasons, about 1/3 of those in summer (Fig. 10). The sterilized cores showed a significant increase in amorphous levels during the fall ($p = 0.00$) when compared with summer values, but dropped to the spring to values similar to the summer. Sterilized core concentrations tended to increase in the spring, but were not significantly different than in the summer (Fig. 10).

Net nitrification rates showed trends similar to the concentration changes; they were low in the field cores in the fall and spring, and were similar or higher (as much as 18.7 μg at 80g-m) in the laboratory cores for the same seasons (Fig. 10).

Twenty-nine-year-old plantations. For the 29-year-old plantation similar trends to the other locations were demonstrated; a significant ($p = 0.00$) decline in amorphous concentrations in the fall and spring for the initial conditions when compared with the summer, and a significant ($p = 0.00$) increase in the same periods for the laboratory cores (Fig. 11).

Net nitrification rates reflected patterns of amorphous concentrations (Fig. 11), except in the field cores where no differences among seasons were observed. In the laboratory cores, a very strong increase ($p = 0.00$) in rates was observed in the fall and spring when compared with the summer.

When the NFI fertilized site was included in the statistical analysis of the 29-year-old plantation, there was a significant ($p = 0.00$) three-way interaction (treatment-seasons method-season, Fig.

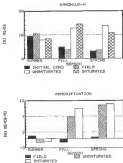


Figure 11 Soil aerobic & concentrations and ambiotized rates to the 10-year-old plantation for the various seasons and incubation methods.

12-13). In general, the following trends were determined when the BPE treatment was compared with the other treatments, or among the incubation periods within the various seasons:

- 1) There was a 3-fold decrease from summer to fall (significant at $p = 0.05$) in the initial ammonia-B concentrations on the BPE site following the August 1981 fertilization.
- 2) There was a 3-fold decrease ($p = 0.050$) in ammonia-B concentrations from fall to spring after another 4 months had passed.
- 3) Net nitrification rates were high in the field and incubated cores during the fall, with average rates ranging from 10.5 to 16.5 mg N/kg-so (Fig. 15), while the unincubated cores showed a relatively high negative value of -4.8 mg N/kg-so for the same period. In both cases, fall rates were significantly different ($p = 0.05$) from summer rates.
- 4) Summer nitrification rates were greater than spring rates from 3.8 mg N/kg-so in spring compared to 5-11 mg N/kg-so in summer (significantly different at $p = 0.05$ for the field and incubated cores).
- 5) When the BPE fertilized site was compared with the other treatments in the summer (Fig. 12), ammonia-B concentrations were similar to the initial incubation cores, but they were significantly higher ($p = 0.05$) after the incubation period when compared with the other treatments.
- 6) After the August 1981 BPE fertilization (fall), ammonia-B concentrations were 3-8 times higher than the non-B fertilized plots ($p = 0.05$, Fig. 16).

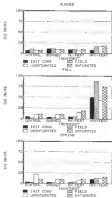


Figure 12. Soil arsenic-B concentrations in the 28-yr-old plantation for the various seasons, treatments, and incubation methods.

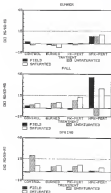


Figure 13. Soft identification rates in the 24-grain classification for the various assays, Unidentified, and Identification methods.

- 7) Ammonification rates (Fig. 11) were statistically ($p = 0.05$) higher in the BPC plots in the summer and fall when compared with rates observed for other treatments.
- 8) During the spring, ammonification rates dropped and were statistically similar to rates observed in the other treatments, except in the associated cores where they were significantly lower than controls (Fig. 11).

Summary

- 1) At the end of the summer incubation period, ammonium-N concentrations tended to be similar for all treatments and plantation ages, although initial concentrations were different.
- 2) Relatively high initial ammonium-N concentrations in summer in the 25-year-old plantation apparently led to relatively low ammonification rates.
- 3) Laboratory incubation cores from the clearcut, 0-, and BPC fertilized 25-year-old plantations collected in the summer tended to have the highest ammonification rates followed by field incubated cores.
- 4) No statistical differences in ammonium-N concentrations or ammonification rates were observed among treatments in the summer, except in the 25-year-old plantation where burned plots had lower values.
- 5) No effect of plantation age was observed in the fall.
- 6) No statistical differences were observed in ammonium-N concentrations between initial conditions and field incubations in any treatment or plantation age in the fall or spring.

- 1) Ammonification rates in the field were significantly lower in the fall and spring when compared with other incubation methods.
- 2) Field ammonification rates were low in all the seasons in the 20-year-old site when not fertilized with NPK, although ammonium-N concentrations at the end of the incubation periods were similar to those found in the other plantations.
- 3) Laboratory incubations were significantly affected by all treatments in the F0, although, in the spring, no significant effects were observed.

Nitrification

Seasonal Effects

Summer: A three-way interaction (plantation age-treatment-incubation method) was detected ($p = 0.001$) for the summer nitrate-N concentrations (Fig. 14). No differences in final $\text{NO}_3\text{-N}$ concentrations were determined in the control or burned plots when plantation ages were compared within each of the incubation methods.

In the PK fertilized plots, field and unsterilized cores in the 5- and 20-year-old plantations tended to have higher final concentrations of nitrate-N than in the clearcut area. Nitrate-N levels in the field cores of the 20-year-old stand were more than 2 and 2 times higher than in the 5-year-old stand and clearcut site, respectively ($p = 0.05$, Fig. 14). For the unsterilized incubation, the 5- and 20-year-old plantations contained about 2 times more nitrate-N concentration than the clearcut area, but they were not statistically significant at the 0.05 level.

Results were variable when treatments were compared within plantation age and incubation method. In the clearcut treatments and

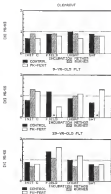


Figure 10. Sperm cell nitrogen concentrations in the various incubation methods, treatments and incubation methods.

*Data available

not have any effect, but in the 5- and 25-year-old plantations, there were some differences. However, no particular trends were observed because, depending on the incubation method, the treatments showed divergent tendencies. For example, field incubated cores from the P2 fertilized plots in the 5-year-old plantation had lower concentrations of nitrite-N than the controls, but in the unincubated and incubated cores, concentrations tended to be similar (Fig. 1A). In the 25-year-old plantation, on the other hand, the unincubated and field cores from the P2 plots had higher nitrite-N values when compared with the control.

When the incubation methods were studied within plantation age and treatment (Fig. 1B), two more results were obtained. First, no significant differences were observed among incubations in the clearest sites. Second, clear trends were observed in the 5- and 25-year-old stands depending on the incubation method. In the 25-year-old stand, the field and unincubated cores collected from the P2 plots tended to have higher nitrite-N concentrations than for the other incubation methods.

In general, no significant trends were observed when nitrification rates were calculated (Fig. 2B), except that all values were very low. It is important to mention that several replications collected in this period were lost due to chemical analysis problems.

Fall. When fall nitrite-N concentrations were studied, only the main effects for incubation method and plantation age were statistically significant ($p = 0.0001$ and 0.14 , respectively, Fig. 2C). Within incubation method, the unincubated cores had about 3 times more nitrite-N than in the initial condition cores ($p = 0.001$),

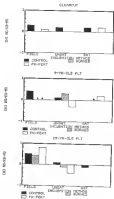


Figure 18. Summer soil nitrification rates to the various plantation ages, treatments and incubation methods

*Non nitrifying

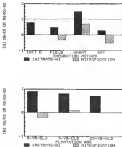


Figure 16. (a) soil nitrata-N concentrations and nitrification rates for the various plantation ages and fertilization methods.

on the other hand, field and saturated cores had less nitrate-N ($p = 0.05$, Fig. 10). The field and saturated cores produced negative values when initial condition concentrations were subtracted (Fig. 10).

Within plantation age, the clearcut site had significantly higher ($p = 0.00$) initial levels of nitrate-N than the 20-year-old plantation, but was not different from the 0-year-old plantation (Fig. 10). No significant differences were observed in nitrification rates (Fig. 11).

Spring. During the spring, an interaction between plantation age and incubation method was found ($p = 0.01$, Fig. 17). No significant differences were observed when nitrate-N concentrations for the initial conditions, field, and saturated cores were compared among stand ages, except for the saturated cores from the clearcut which had nitrate-N levels over 8 times higher than those for the other two areas ($p = 0.05$, Fig. 17). Nitrification rates were also high in these cores, reaching 3.0 mg N/kg-so (Fig. 17).

When incubation methods were compared within plantation age, only the saturated cores collected from the clearcut area showed a statistically significant difference from the other methods ($p = 0.01$, Fig. 17); unsaturated values were at least 8 times larger. In addition, a strong increase in nitrification rates was observed (Fig. 17).

Plantation Age Effects

Summer. Nitrate-N concentrations were also compared by season within each of the stand ages studied. Concentrations from the clearcut area increased significantly in the unsaturated

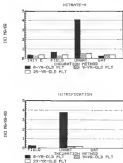


Figure 17 Spring soil nitrate-N concentrations and nitrophosphate rates for the various plantations ages and incubation methods

laboratory cores (Fig. 18). In the fall they were 2 times larger ($p = 0.00$), and in spring they were 4.5 times larger ($p = 0.00$) when compared with summer values. The saturated incubated cores had the lowest concentrations in fall and spring, but were similar to the other cores in the summer.

Minification rates in the cleared site (Fig. 19) behaved similarly to the nitroben concentrations: the unsaturated cores tended to have the highest rates in the fall and spring seasons. In addition, relatively high negative values were observed in the fall for the field and saturated cores which may indicate that denitrification occurred.

Five-year-old plantation. In the 5-year-old plantation, spring nitroben concentrations were lower than in summer (Fig. 18), differences were significant ($p = 0.00$) in the initial conditions and unsaturated cores. Minification rates (Fig. 19) tended to be higher in the unsaturated cores in the fall. In the other cases, almost no changes were observed, except for the saturated cores where relatively high negative values were detected.

Twenty-five-year-old plantation. In the oldest plantation, the spring nitroben values were also lower than those in the summer (Fig. 20), and were significant ($p = 0.00$) for all incubation methods. Fall values were also lower in the field and saturated incubated cores; the decrease was significant at the 0.05 level and values were around 1/3 of the summer values (Fig. 20). Unsaturated cores, on the other hand, had higher values in the fall ($p = 0.00$), but dropped in the spring ($p = 0.00$) when compared with the summer.

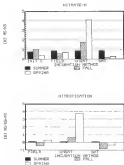


Figure 18: Total nitrate-N concentrations and nitrofication rates in the clarinet site for the various seasons and flocculation methods.

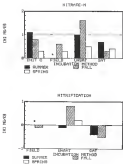


Figure 18. Soil nitrate-N concentrations and nitritification rates in the Sycamore plantation for the various seasons and incubation methods.

*Non estimate

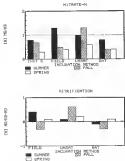


Figure 20. Soil nitrate-N concentrations and nitritification rates in the 20-year-old plantation for the various seasons and incubation methods.

Nitrification rates (Fig. 26) tended to be near 0 or negative for the majority of the cores, except for the field cores in the summer and the unsterilized cores in the fall when values were relatively high (more than 0.4, mg N/kg-mo). No differences were observed in the spring.

When the BPS treatment was added to the statistical analysis of the 28-year-old plantation, a 3-way interaction [treatment-location season] was significant ($p = 0.000$), Figs. 23-25. In the summer, no differences were determined among the treatments, but in the fall, after the fertilization, the BPS plots had very high concentrations of nitrate-N relative to the other plots (Fig. 23). This was also reflected in the fall nitrification rates (Fig. 26) where the BPS plots had much higher rates than the controls ($p = 0.00$) for the field and unsterilized cores (18.8 and 5.9 mg nitrate-N/kg-mo, respectively). The sterilized cores, on the other hand, had a negative value of -26.2 mg N/kg-mo (Fig. 26). This result was expected as aseptic conditions preclude nitrification of the nitrate-N already present and inhibits further nitrification.

In the spring, the only significant effect was that the unsterilized cores from the BPS treatment had much higher values (Fig. 23).

When the location methods were compared within the BPS fertilized treatment, no statistical differences in the nitrate-N levels were found in the summer period, although field cores had double the concentration of the other cores. In the fall, the field and unsterilized inoculated cores had higher concentrations of nitrate-N (Fig. 23) with a large portion extracted from the lower ion exchange

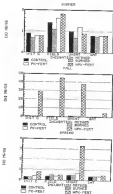


Figure 21. Seasonal soil nitrate concentrations in the 25-year old plantation for the various treatments and incubation methods. Note change in scale.

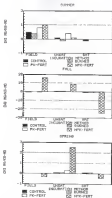


Figure 72. Overall soil nitrification rates to the PK-pert plantations for the various treatments and incubation methods. Note change in scale

nitrate-N in the case of the field cores (see Appendix II). [All comparisons were significant at $p < 0.05$ relative to the initial concentrations]. The saturated cores had about 8 times less nitrate-N when compared with the initial conditions levels ($p < 0.01$). In spring, nitrate-N levels were back to values similar to those found in summer, except for the unsaturated laboratory incubations where values were still more than 4 times larger ($p < 0.05$) than for the other incubations. Nitrification rates were also significantly ($p < 0.05$) higher in the unsaturated cores (Fig. 22).

Summary

- 1) No effects of treatment on nitrification were observed, except for the 80% fertilized plots which had greater nitrate-N concentrations and nitrification rates in the fall.
- 2) There were no effects of plantation age, except for the clearest [0 age] site which had greater nitrate-N concentrations and nitrification rates in the fall and spring.
- 3) Unsaturated laboratory cores showed the highest nitrification rates compared with the other incubation methods.
- 4) A strong decrease in nitrate-N concentrations was observed in the saturated laboratory cores in the 80% fertilized plot after the incubation period, following rapid denitrification of nitrate-N.

Potential Non-symbiotic Biological N₂ Fixation

A significant ($p < 0.001$) three way interaction between plantation age, treatment and season was found when potential non-symbiotic biological nitrogen fixation rates were analyzed (Fig. 23).

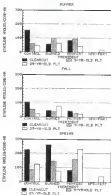


Figure 13— Potential non-typhoidal aluminum fluxion rates (amylase reduction) in the various glassball ages, treatments and seasons

Plantation Age Effects

When plantation ages were compared within seasons and treatments, the following results were obtained (Fig. 10):

- 1) Control areas from the clearcut area had higher (at least 2 times larger) rates than the controls of the 8-year-old and 25-year-old plantations. These were significantly different ($p = 0.05$) in the summer, but, in the fall, only the 25-year-old and, in the spring, only the 8-year-old plantations were significantly lower than controls.
- 2) In the burned plots, summer and fall rates in all the plantations were statistically similar. But in the spring, the cleared rates were about 2 and 3 times higher than those from the 8- and 25-year-old plantations, although they were significantly different ($p = 0.05$) only when compared with the oldest plantation.
- 3) In the FC plots, no differences between plantation ages were obtained for the summer or fall. In the spring, the 25-year-old plantation had rates 2.7 times higher ($p = 0.05$) than the clearcut area.

Treatment Effects (Clearcut, FC, Burn)

When treatments were compared within plantation age and season, no differences were observed in the summer or fall, except in the burned plots from the clearcut where they were significantly lower than controls in the summer, and in the burned plots from the 25-year-old plantation where they were significantly higher than controls in the spring (Fig. 10). During the spring, rates from the control and burned plots from the clearcut area were at least 2.7 times higher

than those fixed in the PI plots, and they were statistically different ($p = 0.05$). In the 8-year-old and 28-year-old plantations, PI plots had higher rates when compared with the other treatments, but were significantly different only in the 28-year-old plantation when compared with the burned plots (Fig. 23).

Seasonal Effects

When seasonal rates were compared within plantation age and treatments (Fig. 25), the following results were obtained:

- 1) The control plots from the clearcut and 8-year-old stands had statistically similar rates among seasons, although there was a 50% drop in the clearcut during fall. In the 28-year-old stand, on the other hand, spring rates were at least 2.7 times larger than summer and fall rates ($p = 0.05$).
- 2) In the burned plots from the clearcut area, spring rates were about 4.2 and 5.1 times larger than the summer and fall rates ($p = 0.05$). In the 8-year-old stand, spring rates were also larger ($p = 0.05$) than fall but were statistically similar to summer rates (about 4 and 1.4 times higher, respectively). No changes in seasonal rates were found in the 28-year-old stand.
- 3) Rates in the PI-fertilized plots from the clearcut area showed no seasonal differences. In the 8-year-old plantation, fall values were significantly lower ($p = 0.05$) than summer or spring values, and in the 28-year-old stand, spring rates were 18.4 and 5.2 times larger than fall and summer values, respectively ($p = 0.05$).

PI Fertilization Effects

A separate statistical analysis was performed in the clearcut plantation to determine potential nitrogen fixation trends when the

the treated plots were included. A significant ($p = 0.04$) treatment-season interaction was obtained.

In all seasons, the NH₄ core rates were consistently lower when compared with the other treatments, and they were significantly different when compared with the control core rates in the fall and spring ($p = 0.00$, Fig. 10). No statistical differences were observed when NH₄ core rates were compared among the seasons, except that spring rates were 2 times higher than the fall rates.

Substrate Effect

Some preliminary studies were performed using Tritic hordei and decomposed wood from the 28-year-old plantation (Table 1). No statistical analysis was made on the data and only three replicates per substrate were collected. Results indicated very high activity in the decomposing wood as opposed to the Tritic hordei, which was 10 times less active. Rates in the wood declined in the spring (March) when compared with the summer (August) values.

Summary

- 1) Control plots from the cleared site tended to have higher potential nitrification rates than the controls of the other plantations.
- 2) Fencing and N₂ fertilization apparently had a negative effect in the cleared site, but had no effect or had a stimulatory effect in the other plantations.
- 3) In the 28-year-old plantation NH₄ fertilized plots had consistently lower potential nitrification rates than the other treatments.
- 4) Potential N₂ fixation rates were highest in the summer and spring,

Table 4. Seasonal potential non-specific R_p fixation (moles nitrogen/m²/year) in the 20-year-old plantation for the various substrates. Standard deviation in parentheses

Substrate	SEASON	
	Summer	Spring
Litter	—	6.2 (1.7)
Wood	831.4 (205.7)	118.3 (100.5)
Mineral soil [control plots]	82.8 (26.8)	46.2 (20.4)

- 3) Very high variability in working reduction was observed, especially in the spring.
- 4) Very high rates of fixation were observed in decaying wood compared with those of the soil. (Essentially no activity was detected in the litter).

DISCUSSION

Ammonification

The amount of nitrogen available for vegetation uptake is largely dependent on mineralization rates, especially in unfertilized areas (Garcera and Nann, 1980; Porter et al., 1984). Net rates are controlled by a series of complex relationships between flotic factors, such as species composition (various plant species may stimulate or inhibit soil N reactions through production of secondary chemicals) and organic matter quality (nutrient concentrations, lignin concentrations, etc.), and abiotic factors, such as temperature, moisture content, nutrient availability, and pH. Because mineralization is a microbially mediated process, small variations in one or more of these factors can lead to very different observed rates.

In the present investigation I found that ammonification rates were variably affected by plantation age, season, treatment, initial ammonium-N concentrations, and incubation technique.

Plantation Age Effects

During the summer (Figs. 3-5), ammonium-N concentrations and net ammonification rates varied among plantation ages-- very low ammonification rates or immobilization of N were observed in the oldest plantation, even in the laboratory incubated cores. At first glance, and when compared with the two younger areas, this would appear to indicate an inhibition (such as by allelochemicals) unique

to the old stand. But when total ammoniac-N concentrations were studied, at the end of the incubation period all the sites had similar concentrations, although initial concentrations varied by a factor of 2. These results suggest that:

- (1) In the old stand, ammoniac-N production occurred earlier in the summer and that uptake under forest was low so that, even with no further ammonification, final N concentrations were similar to the younger stands.
- (2) Once a certain level of ammoniac-N was reached, further ammonification ceased. This may be related to limitation in the availability of C or some other substrate.
- (3) Initial ammoniac-N levels in the younger stands were lower than the old stand. This may be related to higher N losses through leaching (Marsch, 1981), greater uptake rates earlier in the summer, or greater nitrification in these areas.

The clearcut area and the 5-year-old site behaved very similarly, although the 5-year-old plantation had slightly higher net ammonification rates and lower initial concentrations. For these two areas, maximum average ammonification rates were about 5.8 mg N/kg-mo (8.1 kg N/ha-mo), and average total $\text{NH}_4\text{-N}$ concentrations were less than 28 mg N/kg (18 ± 3 kg/ha).

It is important to note that the clearcut area was part of the 25-year-old plantation before the area was harvested in March 1980, only three months before the current experiments were established. Nonetheless, net ammonification rates were 2-3 times higher in the clearcut when compared with the undisturbed stand. Although initial concentrations of ammoniac-N were slightly lower in the clearcut area,

which may have had some effect, the intact stand appears to exert some control over the mineralization process, directly through accumulation of litter or greater nutrient competition, or indirectly through microclimatic or hydrologic changes (Petersen et al., 1975; Petersen and Morris, 1978; Morris, 1980; Nelson and Petersen, 1981). Burger (1980) and Morris (1980) also found increases in N mineralization rates in similar sites after clearcutting, which were directly related to intensity of site preparation.

It has been suggested that N losses may be low in disturbed areas due to rapid incorporation of inorganic N into decomposing organic matter, especially in temperate areas (Vitousek, 1980; Parton et al. 1981). However, on the average, I observed no net N immobilization in any of the incubated cores from the clearcut area, which may indicate that significant losses of N can occur. In this respect, Morris (1981) reported increases in inorganic N concentrations in lysimeter solution collections in the disturbed areas he examined, which, in the absence of vegetation, may indicate N losses to the atmosphere.

In the fall, no statistically significant differences were observed among plantations for either initial concentrations of $\text{NH}_4\text{-N}$ or net nitrification rates. But in the spring (Fig. 8) a significant interaction with plantation age was again observed. During the spring, reduced concentrations and nitrification rates were detected in the clearcut area. As suggested by Stone and Fisher (1983), this may have been due to the absence of canopy in the area, which apparently can lead to stimulation of mineralization (as also has

and Feb. 1989). Also a year after the harvest, less favorable conditions would exist for microbial activity, such as an increase of detritus with higher wood content, less availability of essential nutrients, or reduced input of labile organic C (Balmer, 1981). This could lead a reduction in available ammonium-N (there is also some indication that nitrification rates increased in the spring as will be discussed later).

In addition, it is important to note that during the fall and spring seasons, cores collected from the old stand and incubated in the laboratory, showed relatively very high rates of ammonification when compared with newer (Figs. 8 and 11). Initial concentrations were low in both cases, which reinforces the idea that ammonification rate may depend on initial ammonium-N levels prior to incubation.

In general, all cores tended to have similar ammonium-N concentrations at the end of the incubation period, that is, ammonification rates tended to maintain a certain level of ammonium-N in the soil (10-15 mg N/kg). The differences in plantation age and degree of disturbance, therefore, were apparently insufficient to produce major changes in this nitrogen process during the year investigated. In some respects, it is surprising that, in spite of the drastic differences in soil temperature, soil moisture content, vegetation structure, forest floor organic matter, and possibly nutrient availability after the stand was harvested (see Pritchett and Harris, 1987; Gholz, Fisher, and Pritchett, in press), no apparent immediate effect was detected. Yet, as suggested by Hansen (1989), these forest soils may have some resistance to alterations produced in the site, especially when the area is not intensively prepared.

(Sjögår, 1975; Morris, 1981; Morris and Friedland, 1982). Vianna et al. (1979) also suggested that in some cases a lag may occur before changes in N processes and pools are detected, especially if sites are low in fertility.

Treatment Effects

Treatments also had some effect on ammonification rates in certain instances. For example, when treatments were applied in March, the burned and PK fertilized plots in the summer had lower ammonification and ammonification rates when compared with the control plots (Fig. 5-6) but in the fall, the burned and PK fertilized cores incubated in the laboratory showed a significant stimulation of ammonification, with the PK fertilization having a larger effect (Fig. 7), rates increased almost 2 mg N/kg-so (2.3 kg/ha-so) when compared with the controls. In the spring, no treatment effects were apparent.

It was expected that by burning the forest, ammonification would be stimulated due to possible increases in pH, nutrient, and energy source availability, and by the possible decrease in carbon accumulated in the forest floor (Fire, 1974; Nelson, 1975; Sjögar, 1979). However, it now seems likely that after 8 years or more of no fires in the areas studied, the light here was not sufficient to produce any significant changes. When several soil characteristics were analyzed in the treated plots (Appendix 2), little or no differences compared to controls were determined. Sjögar (1979) reported some changes in stander soils which were prescribed burned, but he also found that the effects were small and temporary, usually lasting a year or less.

Jones and Richards (1977) suggested that once soil increased in pH after a fire may reduce fungal populations in coniferous forests leading to reduced N immobilization, and Christensen (1971) reported a general increase in N mineralization in a California chaparral ecosystem after burning. Other investigators have also indicated such a stimulation (see review by Nelson, 1979). On the other hand, Wells (1971) and Fire (1974), for example, have reported negative effects of prescribed burning on N mineralization. Decreases may be related to a drop in the size of soil N pools versus N mineralization, or to adverse effects of the fire on substrate quality, such as a reduction in hydrolysable forms of N in the soil (Fire, 1974; Jones and Richards, 1977; Baker and Henderson, 1981).

According to Gley and Hall (1961), P and N cycles may be interrelated due to the large energetic requirements of nitrogen processes; N processes will be controlled by availability of P. This would be especially true in P poor soils as in the present study. Monser and Wilson (1977), Ray and Bridge (1979), and Taylor et al. (1980) found increased nitrification rates with greater availability of soil P. On the other hand, Ryan et al. (1977) and Ryan and Sims (1979) found that, in some cases, addition of phosphate reversed the process and led to increased N immobilization due to an increase in microbial population growth.

In the present investigation, mixed results and relatively short term effects were observed when P was added. I found a reduction of soil nitrification rates in the summer, a stimulation in the fall, and no apparent effect in the spring. When P was first added in the summer, microbial populations may have increased with utilized soon

of the available N for their growth. In the fall, when additional P was applied, such an increase may not have occurred permitting the detection of larger levels of ammoniac after incubation when compared with controls. However and Griffin (2022) also observed that, in some instances, relatively low levels of P decreased N mineralization rates, especially in short incubation tests, but when more P was applied to the same soil the results were reversed. They concluded that in order to maximize net N mineralization rates and reduce time lags, large quantities of P were required. In the spring, I found no effect maybe due to the fact that extractable soil P also decreased (Appendix 1), which may have reduced microbial activity.

Finally, the 80% fertilized site in the 26-year-old plantation showed a very large enhancement of net ammonification in the summer and especially in the fall after the direct application of fertilizer (Figs. 10-11). Even when soil's ammoniac concentrations were relatively high, average ammoniac-N production was at least 20 mg N/kg-soil (1 kg N/ha-soil) in the summer, and 25 mg N/kg-soil (25 kg N/ha-soil) in the fall. A nitrate rate of 20 mg N/kg-soil (about 40 kg/ha-soil) was reached in the field incubated units in the fall. A large portion of the ammoniac-N detected was trapped by the lower tree exchange roots (see Appendix 1), and may have been a result of relatively labile organic nitrogen, immobilized after the fertilization, being retransformed microbially with the aid of drying and wetting cycles.

Summer ammonification rates in the 80% site were at least 10 times higher than in the other treatments in the same plantations, but ammoniac-N concentrations only differed about 2 times or less at the

end of the incubative period). On the other hand, fall ammonium-N concentrations were about 18 times larger in the 5% plots, but nitrification rates were only 2.5 times larger when compared with the other treatments. In spring, nitrification rates were low, even lower than in the other treatments, but nitrate concentrations were still high and similar to summer levels. Also (and as discussed in Appendix 4), a significant drop in P concentrations to control levels was observed at this time, which may indicate some influence of P availability.

The results observed above for the summer and fall are in accordance with some other investigations. For example, Ransom and Wilson (1977), Aggarwal (1981), Jones and Whitwell (1984), and others have found increases in N mineralization after sites were fertilized with N, P, and K. They have suggested that such increases are due to a decrease in C/N ratios in the soil (see also Jacobs and Bernier, 1984) and to increases in available P for enhanced microbial growth. Furthermore, as in this case where the A horizon soil was studied, the predominance of organic matter released to mineral soil ("heavy fraction" organic matter, as opposed to the the "light fraction") may have contributed to increases in net N mineralization, as proposed by Jallin et al. (1984), by decreasing the availability of C to be used as an energy source by microbial organisms. In other words, the limiting factor for microbial growth was C instead of N, so that less N was immobilized (see also Jacobs and Bernier, 1984).

Incubation Method Effects

When different incubation methods were studied, several tendencies were established:

- 1) The saturated laboratory incubated cores had the highest initial ammonium-N concentrations and nitrification rates, except in the summer for the cores collected in the 20-year-old plantation. Senney (1980, 1982) indicated that, in general, this type of anaerobic incubations usually yields higher levels of ammonium-N than aerobic incubations, and that ammonium-N released tends to correlate well with plant uptake and growth (see also Fosses, 1982). Results from my incubations suggest that in the field, when an area floods, ammonium-N may accumulate due to reduced nitrification rates, and, perhaps, reduced plant uptake.
- 2) Unsaturated laboratory incubated cores followed similar patterns as the saturated cores, although ammonium-N concentrations tended to be lower at the end of the incubation period. Although nitrate levels were also low, as will be discussed later, some of the ammonium-N nitrified may have been nitrified and subsequently denitrified in high humidity microsites (Senney, 1982). On the other hand, the moisture content of the soil cores may not have been optimal in all the cases, affecting the nitrage nitrification rates obtained (see also Cassan and Hays, 1982).
- 3) The field incubated cores tended to behave similarly to the saturated cores in the summer. This similarity may have been due to the high humidity conditions found in the field, in several instances (at least three times during the incubation period) the sites flooded. Yet, in the fall and spring little or no differences were detected between the field incubated cores and the initial concentrations, although the laboratory cores showed relatively high activity. This suggests that the lower soil

temperatures compared with the summer were an important factor in regulating the N mineralization process by reducing microbial activity. There is some other evidence that this may be the case. For example, CO_2 respiration measurements made in the same area (Shen, Jari, Cropper, and Hendry, in press) show that evolution decreased in August-September and increased again between April-May, and was highly correlated with changes in soil temperature. In addition, other biologically-refracted, variables, such as changes in P and soluble organic carbon availability, decreased during the same periods (see Appendix 1 and 4). Tate and Meyer (1984) also found decreased soluble organic C concentrations in streamwater, during the winter from several forests in the Eastern Basin, North Carolina.

Results from my study indicate that, although the potential to mineralize nitrogen existed all year in the laboratory incubation trials, in situ conditions did not allow the process to occur in the fall and spring.

Finally, single physical or chemical variables measured strongly influenced nitrification rates. The most important variables were C_org , NO_3^- , pH, and soil moisture content, but their ability to improve regression equations was small, the r^2 for the most complete linear model was only 0.36.

Nitrification

Nitrate concentrations in forest soils are usually low for a variety of reasons (Vitousek et al., 1987), such as low nitrification rates, rapid immobilization of NO_3^- by vegetation, and rapid losses NO_3^- through leaching and/or denitrification.

According to several workers (see review by Seeny, 1980) most forested forests show low nitrification rates because of the acidic conditions usually found in the soil. Burger (1976) determined that, for nature pine stands similar to those of the present investigation, a nitrification threshold for pH was around 4.5. Surface soil pH in the present investigation area tended to be around 4.8 (Appendix 2, Bels, Fisher, and Frischbitt, in press). Other potentially important factors include the presence of inhibitory allelochemicals (see review by Kim, 1980), or the low availability of substrates such as ammonium-N and biogenic P (Christie, 1981). In addition, if ammonium availability is low, heterotrophic bacteria are considered to be better competitors for $\text{NH}_4\text{-N}$ than nitrifying nitrifiers (Jones and Richards, 1977), so that nitrifiers will be outcompeted even if other environmental factors are adequate.

Plant uptake of nitrate may also lead to low nitrifications. Nason (1977) and others have concluded that vegetation in early successional communities preferentially uptake nitrate, while in older communities ammonium-N is preferred. In addition, Kang and Oak (1980) observed that the coniferous forest they studied absorbed less nitrate than a nearby beech forest. In the other hand, Artwell and Adams (1984) determined that if nitrate levels were high, nitrate reductase activity in roots of *P. maritima* B. var. *maritima* increased.

If little plant uptake occurs, and if the soil has a low cation exchange capacity, as many forest soils do, then nitrate losses through leaching or denitrification are expected to be high.

My results indicate that very low nitrate concentrations and nitrification rates occurred in the slash pine plantations, especially

in the fall and spring (concentrations were usually less than 0.5 mg N/kg or 1.1 kg N/ha, and rates less than 0.5 mg N/kg-soil or 0.6 kg N/ha-soil). Similar results were found by Morris (1981) and Burger (1979) in sites comparable to the ones studied here. I did observe some variations in results, however, depending on treatment, plantation age, season, and incubation method.

Plantation Age Effects

Plantation age had some effect on the nitrification process, but varied depending on season. During the summer (Fig. 14-15), nitrate levels were higher in the 9-year-old plantations and the P0 plots of the 20-year-old stand than in the clearcut area, but no other differences were detected. No major variations were observed in nitrification rates, although they were slightly higher in the P0 plots of the 20-year-old stand. Most values were less than 0.1 kg N/ha-soil.

During the fall (Fig. 16) a small but significant increase in nitrate concentrations was found in the clearcut area, although overall nitrification rates were lower. In the spring (Fig. 17), nitrate-N concentrations and nitrification rates were greatest in the clearcut. This agrees with results of Wilson and Steward (1980) who found that nitrification increased after an area was disturbed, although in my study it began to rise only a year after the site was harvested. American and inorganic P levels in the clearcut site were similar or lower in spring than in the other two seasons, which suggests that rather than a nutritional limitation (such low available P), as indicated by Ardhouse (1978), Bernstein (1981), and Wilson et al. (1982), other factors were regulating nitrification. Nit's ex-

point to a decrease in allelochemical concentrations because of removal of the plant inventory as suggested by Hise and Pandey, (1992).

As mentioned, nitrification rates were high in the clearcut in the spring, a year after the site was disturbed. This lag in response would reduce nitrogen losses allowing time for vegetation to recover (Vitousek et al., 1987), although I expect that, with higher temperatures and a still undeveloped vegetation in the summer, leaching losses would still be relatively high.

In the 9-year-old stand, with a strong herbaceous understorey, no differences were observed with season and were similar to results observed in the 25-year-old stand. This indicates that increases in nitrification rates which could occur after a disturbance, decrease early as stands develop.

Fireproof Effects

Burning and PK fertilization did not have a strong effect on nitrification. In the burned plots, no changes in soil chemical characteristics that were observed (Appendix 2) apparently had any effect on nitrification. Instead, Water B pools may have been reduced through volatilization which increased heterotrophically bacterial competition for the remaining available-N substrate. On the other hand, P and K availability were increased in the fertilized plots, but still, no effect on nitrification was detected. (except for a small and temporary increase in the summer in the 9- and 25-year-old plantations). Although Potholke (1974) and Pastor et al. (1991) suggested that aqueous P availability limits nitrification, in my

established stands other factors seem more important, such as low available ammonium-N or accumulated toxins.

There is some indication that when ammonium-N levels are increased, in conjunction with adequate levels of available P, nitrification rates may increase. For example, when the 28-year-old plantation was fertilized in August with NPS (30 kg urea), fall incubated cores showed high concentrations of nitrate and high nitrification rates. After the one month incubation, nitrate concentrations rose 5 to 40 times, depending on incubation method, when compared with other treatments (Fig. 21), and rates increased from negative or near 0 values to a maximum of 15 mg N/kg-so (approx. 38 kg/ha-yr) in the NPS field incubated cores (Fig. 22). These results are in accordance with those of other investigators such as Williams (1932), Robertson (1964), and Adams and Whitfield (1968), who also reported increases in nitrification with added nitrogen. However, a climate does not always occur (Johansen and Cowley, 1969; Brown, 1975; Reiser et al., 1982; Aggarwal, 1984). Some reasons for such apparently conflicting results are that there were differences among studies in substrate quality, the type and amount of fertilizer applied, the effect of the fertilizer on pH or other soil factors, the availability of other nutrients, and the delay between the treatment application and the incubation of the studies. Brown's (1975) site was very similar to my sites. When he fertilized a 28-year-old slash pine plantation with urea, the soil pH increased by two units. But another factor appeared to stimulate nitrification, indicating that perhaps low availability of P was controlling the process.

The high nitrification rates I found in the 28-year-old plantation when NPK was added, appear to confirm the hypotheses and results of Link (1980), Yavinecova (1975), Nelson and Stouffer (1981), and Robertson (1980) that nutrient availability, rather than plantation age, successional stage of the forest or the presence of a toxin, was the factor controlling this process. However, the fact that fertilization increased soil pH, and that the control site was fertilized with these chemicals three years in a row, may have reversed existing acidity and toxin inhibition effects and permitted an increase in nitrifier population growth (Bock, 1971).

Isolation Vessel Effects

- The results of the various isolations showed that, in general:
- 1) Unstarved laboratory cores tended to have the highest concentrations of nitrate-N. Concentrations were especially high in the fall and spring in the NPK fertilized cores from the 28-year-old plantation, and in the spring in the clearest area.
 - 2) Plate isolated cores had relatively low nitrate-N concentrations, except for the NPK plots. Much of the nitrate-N from the NPK cores was trapped by the lower ion exchange resin (Appendix 1). Some unstarved denitrification may also have occurred as not all the nitrate-N may have reached the resin during the 30 days and because the sites flooded several times during the incubation period, providing an environment conducive to denitrification.
 - 3) Starved cores also have low levels of nitrate. This was expected because nitrification ceases under such high sulfide contents, and the nitrate-N present will be denitrified (Kenney, 1982). Kelly and Rao (1982) suggested that even nitrification can

still present as ammonium-N diffuses to microsites relatively high in oxygen. In my study though, flood water was not aerated and very acid, and available ammonium-N was low.

The drop in nitrate-N was especially pronounced in the saturated cores after the 1982 fertilization at the 20-year-old stand. A negative value of 24 mg N/kg-soil (36.4 kg N/ha-soil) was obtained, representing a nitrate loss of approximately 5.5 kg/ha-day when the soil was saturated. Such a decrease in nitrate concentration did not occur in the field incubated cores for several reasons: nitrification was possible when the cores were not saturated, nitrate was leached down and trapped by the lower ion exchange resin where no further transformation occurred (Nelson¹, 1982), and field cores were not under high moisture contents for much of the incubation period.

In general, a drop in nitrate concentrations and nitrification rates occurred in all the sites in the fall and spring, except in the cleared area and the 1982 plots (in the fall only). This may have resulted from a decrease in available ammonium-N and a decrease in microbial activity in these periods (as was discussed in the Ammonification section). Regression analysis showed some influence of ammonium-N concentrations on nitrate levels in the soil cores at the end of the incubation period, as well as of other factors such as pH, Al, Ca, and soil moisture content, but the amount of with all factors included was equal to 0.13.

Finally, the effect of urea fertilization on nitrification was temporary. When cores were incubated in the spring, less than four months after the fall incubation, nitrate levels were very similar to those in the control plots (Fig. 82).

Potential Non-Symbiotic Biological N_2 Fixation

Nitrogen inputs to forests through non-symbiotic fixation are considered to be low when compared with symbiotic fixation or when compared with total existing N pools. Nonetheless, as it stressed ecosystems, even small amounts may be important to the overall stability of the system.

Forest soils are characterized by low pH's (Boney, 1980) and this is certainly the case of many floridic forests in Florida (Peterson and Garberd, 1982). According to Jørgensen and Boney (1984), this condition tends to favor the development of the free-living bacteria Chlorobium sp. and Thiobacillus sp. In addition, Brown et al. (1980) found that, in some Quebec forest soils, greater N_2 fixation usually occurred under anaerobic environments and that Chlorobium was widely distributed in these soils. Graham and Lindberg (1970) found that, in stands of Scots pine and in stands of alder pine and Norway spruce, heterotrophic nitrogen fixation was mainly due to anaerobic and facultative anaerobic bacteria, but was greatest in sites with high C/N ratios, relatively low N_2 tensions and low acidic conditions. Olsche and Howarth (1972) indicated that in some sandy pine forest soils, low nitrogen fixation efficiencies of aerobic bacteria under low oxygen concentrations were caused by intense competition for energy sources and by low pH's. Lajtha and Nelson (1970) have suggested that pine forests tend to inhibit the development of aerobic nitrogen bacteria while encouraging anaerobic ones.

On the other hand, Janda et al. (1982) found that in Florida agricultural soils, fixation activity was favored by high relative

contents and the sugar concentrations (the nitrogenous sugar properties are destroyed in the presence of oxygen) and that Glucosella sp. and Enterobacter sp. bacteria were the most predominant [R. Smith, pers. com., 1944].

Other nitrogen fixing organisms, that have been associated with forest soils, include the blue-green algae (Cyanophyta) which may be of great importance in cleared or open stand areas [Jungersen and, Insay, 1948; Greshall and Lindberg, 1950]. In addition, associative relationships, have been discovered between coniferous tree roots and N_2 fixing bacteria [Silvester and Jamell, 1951; Richards, 1953] and according to Richards (1953), Glutrizibac was one of the most dominant species. This condition would certainly improve the nitrogen fixation capabilities of the bacteria and the efficiency of trees to absorb the fixed N.

Biogeochemical Fluxion rates reported in the literature for forests are variable. For example, Greshall and Lindberg (1950) determined values ranging from 0.25 to 26.5 kg N/ha-yr, while Good et al. (1954) found values ranging from 15-25 kg N/ha-yr. In general, reported rates are lower than this, usually below 5-8 kg N/ha-yr in g-lades et al., 1950 for different forest systems.

In my study relatively low potential nitrogen fluxion rates and very high spatial variability were obtained for the A horizon (Fig. 2). In Appendix II with a maximum average rate of 251.4 moles of ethylene/acre-hr. This is conservatively calculated to yield approximately 5.2 kg N/ha-yr assuming this rate was constant for a month/year and 10 hrs/day, and with a 5.1 conversion factor (ethylene dinitrogen fixed). This value represents one sixth of the N

uptake requirements of these systems [see Gbure, Fisher, and Frischbatt, in press], average values would be between 2 and 3 kg N/ha-yr.

Stand age, treatment, and season produced mixed effects on nitrogen fixation rates with statistically strong interactions. For example, the control plots from the clearcut site showed higher rates than control plots from the other two plantations. Yet the basal and PE fertilized plots from the clearcut site tended to be lower than the same treated plots from the other two sites.

In the 20-year-old plantations, relatively low rates were detected in the summer and fall regardless of the treatment, but rates increased during the spring in the control and PE plots. In the other hand, the 50-year-old stand tended to have medium to high rates in all seasons when compared with the other two sites.

Higher nodule reduction activity in the clearcut site may have been related in part to higher temperatures, higher moisture contents, higher availability of C sources, and higher light intensities near the soil surface when compared with the other stand. Some of these conditions may have favored free-living nitrogen fixing bacteria as well as blue-green algae (Jørgensen and Søren, 1988; Goodell and Lindberg, 1988), although no evidence of algal crusts were observed. In addition, the presence of grasses, especially in the spring, may have positively influenced potential nitrogen fixation rates. Smith et al. (1981) found that highest activity was associated with large amounts of grass-root, with *Brassica vulgaris* (Brassica) as the most prevalent species, and that corn with no roots showed very low activity (J. Smith, pers. comm., 1984).

Treatments also yielded different functions depending on the season and plantation age (Fig. 23). In the clearest, both burned and PC fertilized plots tended to have lower rates than the controls in all the seasons, except in the spring when activity in the burned plots increased. But, in the 8-year-old plantation, those the two treatments (i.e., burned and PC fertilization) tended to stimulate fixation, especially in the PC fertilized plots. Finally, in the 20-year-old plantation, treatments tended not to be very different from the control plots during the summer and fall, although the burned plots had slightly higher averages. In the spring, PC plots activity increased drastically, but burned plots tended to have similar values as before. Control plots also showed an increase in potential nitrogen fixation activity in the spring, but rates were not as high as the PC plots.

In the NPC fertilized site, consistently lower potential nitrogen fixation rates were observed in all seasons when compared with the other treatments in the 20-year-old plantation. Nitrogen fertilizer addition probably inhibited potential R_p fixation activity, as reported by Ladegren (1988), van Kessel and Berendse (1991), and others. Also lower soil moisture contents tended to occur in this plot (see Appendix B), which may have lead to lower activity.

In the Humbern, various effects of treatments have been reported. Jongsom and Kelly (1971) were some of the first investigators to observe that prescribed burning may stimulate nitrogen fixation activity (in a naturally pine plantation in E. Carolina). They suggested that such an increase was attributable to the release of nutrients, to lower activity levels, and to an increase

in light penetration after the fire. They also found high spatial variability (only 1/3 of the samples showed higher activity in the burned plots) and suggested that this was related to the non-uniformity of the burn. Other studies (Fennell and Stefansson, 1956; Isaac and Shapiro, 1957; Lark, 1961, as reviewed by Isen, 1970) detected increases in *Actinobacteria* and *Chloroflexus* populations after burning. However, other investigators have not found such a stimulus after burning (e.g. Jørgensen, 1976; Nann et al., 1982) which indicates that other factors must be considered when evaluating this effect on nitrogen fixation activity.

Similar mixed results have been found on the effect of P additions on nitrogen fixation, although little work has been done with non-symbiotic organisms. According to Griffith (1984, as review by Cole and Bell, 1987), due to the high energy requirements of the nitrogen fixation process, P availability can control root infection, growth, multiplication, and activity levels of nodules and bacteria in the rhizosphere. Van Kessel and Bakken (1982) observed increases in activity (acetylene reduction) in leguminous nodules when P was added, but the stimulus depended on P concentration and age of the plant. Isen (1970) found at least a 20-fold increase in non-symbiotic fixation rates in a P-fertilized lake, and Silvester (1976) reported a stimulus to activity in the decomposing humus layer of a New Zealand forest after P levels were increased. On the other hand, Jones (1978) failed to improve fixation rates of indigenous nitrogen fixers in a coniferous forest when he added phosphorus alone or in combination with other nutrients.

Time of the year had a relatively strong influence on the nitrogen fixation rates I found. This also has been reported in other studies (see, for example Vance et al., 1983, Herling and Smith, in press). In general, the highest rates occurred in the summer and spring with a relatively large decrease in the fall. Especially high values were detected in the spring, but variability was also higher than in the other seasons.

Several stepwise linear regressions and correlations were performed using the logarithmically transformed arbutane production data to try to link arbutane reduction to variation in several soil chemical and physical characteristics (Appendix X). Results showed very low r^2 values (0-25) even when several factors were included in the model. The complete model was:

$$\ln A = 1.4 + 0.02\ K + 0.10\ OM + 0.08\ MC + 0.01\ Zn$$

where $\ln A$ is the log. transformed value of arbutane produced in the core, K is potassium (g/g), OM is organic matter (%), MC is moisture content of soil (%), and Zn is zinc (g/g). Smith et al. (1984) also found positive relationships with MC and Zn , although the above model disagrees with the work of Smith et al. in that OM and K did not appear in their model. In addition, they found that cation concentrations had some effect.

In summary, mixed results were obtained when potential fixation rates were compared among treatment, plantation age, and season. But, in general, low rates were observed in all cases regardless of the treatment applied or plantation age. Rates tended to decrease with season. In addition, very high spatial variability was observed which

suggests that very specific conditions or factors must interact to yield the higher levels of fixation.

In addition to the soil core study, I performed several preliminary tests on decomposing wood collected from the 20-year-old stand. They indicated that potential nitrrogen fixation activity may be high in certain microsites (Table 4), and may even be higher per kg than in the surface mineral soil. From 4.4 to 12.3 kg N/ha-yr were potentially fixed assuming the forest floor was covered completely with this type of wood and using the time assumptions as above. However, because large wood pieces cover only about 1% of the ground area, actual values would be less than 0.1 kg N/ha-yr. A similar study on the litter layer alone was also made, but very low rates, about 0.1 kg N/ha-yr, were detected.

Relationships to Vegetation

Decreased availability of N and/or P is one of the causes for an early cessation of plantation growth (Friedrich and Gowerford, 1980; Gholz and Fisher, in press; Gholz, Fisher, and Friedrich, in press). In this section, different sources and sinks for N will be discussed and compared to vegetation uptake.

By definition, the total net mineralization rate is equal to the difference between the total amount of decomposable N at the end of the incubation minus the total amount at the beginning of the incubation. Net nitrification in the core is possible (e.g., in aerobic incubations) if nitrate concentration changes also need to be considered.

Based on the field and laboratory incubation results, and if it is assumed that there were between 4 and 6 months of significant activity (no mineralization occurred in the field incubated cores

during the fall and spring] and an average immobilization rate of 8-10 mg N/kg-yr (8-10 mg N/kg-yr), 24 to 32 kg N/kg-yr were immobilized during the year which could have been absorbed by vegetation, nitrified, or lost through leaching.

According to estimates by Shala, Fisher, and Peltisbett (in press) in the same or similar study areas, annual soil N uptake rates of vegetation ranged from 41 kg/kg-yr in an 8-year-old plantation, to 20 kg/kg in a 20-year-old plantation. In their study, it was suggested that nitrification processes in these near sites and the small atmospheric inputs are not able to compensate for decrease availability of nutrients, especially N and P, over time, leading to cessation of biomass increments around 25 years. But, at least in the case of N, the net N immobilization rates calculated in this investigation, indicates that supplies of N are sufficient adequate for stand growth, especially in the young plantations. Nutrient requirements in young stands are greater due to the rapid growth rates and because vegetation is more dependent on availability in the soil rather than on internal recycling, in contrast to older stands were dominated by pines (Shala, Fisher, and Peltisbett, in press). But it is also important to note that only 22% or less of the total inorganic N found in the field cores was trapped by the bottom two exchange resins (Appendix 1), which may indicate that mass flow of N to roots may be a limiting factor.

In older plantations, the forest floor is an active sink for nutrients, especially of N and P (Miller et al. 1979; Shala, Perry, Cropper, and Newby, in press; Shala, Fisher, and Peltisbett, in press), and as the litter layer apparently continues to accumulate in

the chance of having these nutrients still not become available for some time. Also mineralization rates are usually greatly reduced with depth in the mineral soil (see Gassman and Peters, 1988) which suggests that vegetation may be very dependent on mineralization taking place in the surface mineral soil for their N requirements. Additional N sources for plant uptake include throughfall solution, non-symbiotic N_2 fixation. According to Bels, Fisher, and Fredjane (in press), throughfall may contribute approximately 10 kg inorganic N/ha-yr which can reach the mineral soil. By itself, this amount will not be enough to satisfy the plantation uptake rates but will reduce the dependence on net mineralization rates. Values for the 8- and 28-year-old plantations were similar.

Another possible N input is non-symbiotic nitrogen fixation. According to the results I obtained, average rates were below 2 kg N/ha-yr. This is a very small contribution to the total N uptake of the vegetation, but could be important in building the N pools of the ecosystem over a rotation. In addition, point sources of nitrogen fixation in decomposing wood may be important, although rates are limited by the relative scarcity of dead wood in plantations.

A diagrammatic summary of estimated nitrogen fluxes for an unfertilized 28-year-old plantation is presented in Fig. 24. Similar fluxes occur in the other two sites, except that in the cleared area there is less vegetation uptake and, as a consequence, N inputs are increased, and in the 8-year-old stand that vegetation uptake is somewhat higher (around 40 kg N/ha-yr). According to my calculations, vegetation uptake and available N are nearly balanced in the two older plantations, reducing solution N losses from the ecosystem (Morris and



Figure 28. Nitrogen Flows (kg N/ha-yr) for an unfertilized 25-yr-old slash pine plantation (* from Harris and Pritchett, 1982, and from Janda, Ponder, and Pritchett, in press)

Fritsch, 1983). On the other hand, the recent cleared area had higher mineralization rates as the above areas with no evidence of N immobilization, suggesting that, because little or no vegetation was established during the study, there is a high probability that N was lost from the system which reduced N demands for developing plantations and highlighting the importance of other N inputs over the subsequent rotation. The vegetation on these sites may be adapted to selected N mineralization rates with growth patterns adjusted to reduce requirements. Pines, including slash pine, are adapted to infertile soils (Juliusen and Nelson, 1988; Miller et al., 1979; Fritsch and Eversford, 1982) and the following suggestions have been suggested that enable coniferous species to survive under these conditions:

- 1) Pines have low nutrient requirements as indicated by the nutrient levels found in the tree tissues.
- 2) Nutrients are cycled efficiently, losses from the ecosystem are reduced.
- 3) Nutrient inputs are effectively retained through immobilization in the forest floor or vegetation components.
- 4) Pines are able to internally recycle nutrients reducing their dependence on soil availability.
- 5) Pines have special adaptations, such as symbiotic associations, with which the pine trees to obtain otherwise unavailable nutrients.

Due to these adaptations, not all plant species adapted to infertile soils are able to increase their growth rates when availability of nutrients increases. For example, Chapin (1982) found that certain lodge pole tree species tended to accumulate nutrients without

increasing biomass production when the availability of nutrients increased. In addition, some of the adaptations to infertile conditions may not be stimulated and may become a disadvantage when fertility increases, such as the case of the apocarpic association. In slash pine trees, growth responses to increased nutrient availability have been well-documented (e.g., Fisher and Barrett, 1983), although the degree of response depends on age and soil characteristics of the stand. This suggests that slash pine, although adapted to poor soil environments, can adjust physiologically to take advantage of nutrient increases.

Very low levels of nitrate preferential to my plots not fertilized with N, and with few exceptions, nitrification rates were around 0.8 mg N/kg-m or less, suggesting that ammonium-N may be the main N species absorbed by vegetation. But nitrification was high in the 8% plot in the fall, adding a significant amount to the total N accumulated during the incubation (Table 1). As much as 32 mg N/kg-m (30.2 mg N/kg-m) total net nitrification was observed in the 10% incubated cores on this site, 21% of which was nitrate. This high value in the field cores when compared with the laboratory cores, may have been a result of drying and wetting cycles which helped mineralize labile organic N (Birch, 1985; Takeuchi, 1980) and, under unsterilized conditions, nitrification was possible. Denitrification losses of nitrate were most likely prevented or greatly reduced by the ability of the ion exchange resin to trap and preserve nitrate. Analysis of bottom resins (Appendix 1) also showed that the potential for nitrate to leach was high if nitrification uptake for this N form species is low. The unsterilized laboratory cores showed a positive

Table 7. Net changes in soil B concentrations ($\mu\text{g kg}^{-1}$) after a 30-day incubation in the 25-yr-old plantation for the BPS treated plot during the fall, and for the various incubation methods.

	INCUBATION METHOD		
	Field	Soil ₁	Soil ₂
Net B	26.2	-8.0	23.1
Net C _{org} B	15.8	9.0	-24.2
Total B	42.0	1.0	-0.1

balance of only 1 mg N/kg-mo (1.1 kg/ha-mo). Apparently a portion of the ammonia-N was nitrified or denitrified as values tended to be smaller than initial concentrations. If the N assimilation rates were similar to the naturalized or field cores, and ammonia-N nitrified, then a part of the nitrate formed could have reached high biologically accessible levels in the cores where it was denitrified (there is some indication that denitrification is possible as suggested by the associated laboratory results, table 3).

If it is assumed that the total net mineralization of 67.2 kg N/kg-mo from the BPC field cores in the 1977 was equivalent from August, when N₂O was first found, to November, when the incubation was performed, an estimated N production of 228.8 kg N/ha occurred. This value represents about 8 times more N than the vegetation can possibly absorb, so that about 200 kg N/ha may be available for other ecosystem processes or for leaching.

SUMMARY AND CONCLUSIONS

Soil nitrification, nitrification, and potential non-symbiotic nitrogen fixation were monitored in an age sequence of slash pine plantations (0-, 4-, and 16-year-old) in order to evaluate the effects of burning, P and K (0-kg-m⁻² stimulation only) fertilization, and plantation age. In addition, water soluble organic carbon, double acid extractable P, and other soil variables were measured to determine if relationships with the above processes existed. Laboratory, un-aerated (anaerobic) and aerated (aerobic), and field incubated unfertilized (A soil) cores were used to assess nitrification and nitrification rates. The field incubation consisted of a core sealed in which both ends of intact cores were covered with a nylon-weave bag containing an ion exchange resin. This limited diffusion of gases from and into the core, permitted changes in solution content during the incubation period, and avoided desiccation buildup of nutrients in the soil solution (stimulating in part, uptake by vegetation) as they moved down and were trapped by the resin. Biological nitrogen fixation was determined using the acetylene reduction method.

Results obtained from this investigation lead to the following conclusions:

- 1) Ammonia-N concentrations (10-15 mg N/kg) were similar in all sites not fertilized with N at the end of the 30-day incubation period regardless of the initial concentration.

- c) Ammonification rates apparently were affected by initial $\text{NH}_4\text{-N}$ at the beginning of the incubation. If ammonia-N levels were low, ammonification rates were high, in the majority of the cases rates ranged between 5-30 mg N/kg and 8/kg-on for the laboratory incubated cores and field incubated cores in the summer.
- d) Nitrate concentrations and nitrification rates were low in all plantations (less than 1.0 mg N/kg and less than 8.0 mg N/kg -on, respectively).
- e) Nitrogen concentrations and ammonification and nitrification rates decreased in the fall and spring when compared with the summer.
- f) In the recent clearcut no indication of any N immobilization was detected and little or no vegetation was present at the time. These factors suggest that higher leaching losses would be expected to occur in this area.
- g) Nitrification rates in the undisturbed laboratory cores increased in the recent clearcut area one year after the harvest to a level an order of magnitude higher than in other areas. Ammonia-N and inorganic P levels in the clearcut were the same or even lower than in the other plantations, suggesting that nitrification was inhibited in some manner prior to the harvest. This again indicates that losses through leaching and denitrification may increase after cutting.
- h) Ammonification rates were low in the 25-year-old plantations during the summer as determined by all the incubation techniques, but were similar to the other areas during the fall and spring in the laboratory incubated cores. This apparently was related to higher

nitrate] ammonium-N contents in the summer rather than to other factors related to the presence of the forest stand.

- 4) Fertilizing had little effect on ammonification or nitrification. A slight and temporary decrease in ammonification was observed in the summer in all sites, less than 3 months after the fire (especially in the 20-year-old plantation). Such a decrease may have been related to an increase in microbial growth or to a decrease in labile organic N available for ammonification.
- 6) PK fertilization alone had little effect on ammonification and nitrification, although a slight stimulation in ammonification occurred during the fall in the laboratory isolated cores.
- 10) NPK fertilization significantly stimulated ammonification and nitrification in the 20-year-old plantation. This suggests that all three nutrients are required for these processes to occur at near potential rates, although some other factors, such as changes in soil pH and physiology of the vegetation after fertilization, may also have contributed. This stimulation was temporary: less than a year after the fertilization, ammonification and nitrification rates returned to control levels.
- 11) Saturated (aerobic) laboratory cores exhibited the highest net ammonification rates, followed by the unsaturated and field cores.
- 12) High denitrification rates apparently occurred in the saturated cores during the fall in the NPK fertilized plot, as around 60% of the nitrate present prior to the incubation disappeared. Some

non-satisfactory reduction of nitrate to ammonia also may have occurred

- III) Nitrogen concentrations and nitrification rates in the field insulated cores were similar to those found in the saturated cores during the summer, but decreased sharply in the fall and spring. This suggests that field cores were sensitive to on site conditions, mainly due to decreased winter soil temperatures, and that drying and wetting cycles in the cores did not apparently produce a strong increase in available N . Very moist, and often saturated, conditions predominated during the incubation period which may have overwhelmed the effect of drying-wetting cycles.
- IV) Water soluble organic carbon and extractable P concentrations apparently did not affect N mineralization or nitrification, suggesting that quality and not quantity of soluble C , and the availability of P in these sites may be the limiting factors.
- IV) Very low non-biological N_2 fixation rates and high variability were observed.
- IV) Higher biological N_2 fixation rates were detected in the clearest perhaps related to higher surface soil moisture contents, higher temperatures and higher light intensities observed in this area.
- IV) Biological nitrogen fixation could contribute less than 10% to the N uptake of the vegetation.
- IV) In the NRE fertilized site, consistently low biological N_2 fixation rates were observed, perhaps related to relatively high N concentrations in this site.
- IV) Biological nitrogen fixation rates decreased during the fall but increased during the spring relative to the summer.

- [25] Nitrogen uptake rates for the 9- and 20-year-old plantations appear to be in equilibrium with the N mineralization rates observed and to other possible external N sources (e.g., throughfall or biological nitrogen fixation inputs). This suggests that N losses to these sites is minimal. On the other hand, in the chemical sites, losses are expected to be high

APPENDIX I

TOTAL INORGANIC N AND P CONCENTRATIONS IN THE FIELD AND SATURATED LABORATORY INCUBATED CORES

Introduction

In this section, results from the field and laboratory saturated cores will be discussed. Data presented in the main text for the field and saturated incubation techniques represented the sum of N and P concentrations found in both the soil and bottom iron exchange resin (IER) extraction or distilled water solution, respectively.

Only the bottom IER was analyzed. It was assumed that the leachable N and P fraction moved down as rainfall water percolated through the core, before the area flooded. If the site floods, addition of an entire ring with IER at both ends of the core would improve the method and would eliminate the need for these assumptions.

Soil N and P may be desorbed from the soil exchange sites when the core is saturated with water (see Kemp and Rea, 1982). If the water contains sufficient oxygen, part of the NO_3^- -N may be stimulated and lost later by denitrification. In the present investigation, little transformation were expected to occur, as diffusion of NO_3^- is very low, pH's were acidic (5.0 or lower), and water was not aerated. In addition, in the saturated incubations water levels were about 1.5 to 2.0 cm above the soil surface and the top portion of the cores were covered with a perforated plastic layer to reduce ventilation.

Methods

After the 30-day incubation, the soil portion of the field and saturated laboratory incubated cores were analyzed as described. In addition to the soil analyses, the exsudate water from the saturated cores was decanted for 20 to 30 min until no more drops were seen. Solutions were then filtered with Whatman #42 filter paper, brought to a constant volume, and acidified with concentrated HCl before storage at 4° C. Analysis was performed 3 to 5 days after, except in the summer phase where it took longer. Magnesium S and P were analyzed using the Technicon Autoanalyzer II.

Sodium IRT's from the field cores were air-dried to a constant weight, which took about 8 to 10 days. Each active IRT in the cellophane bag was then weighed out 2 g mixed with 50 ml 2N HCl for 1 hr. The mixture was filtered with a Whatman #42 filter paper and analyzed with the Technicon Autoanalyzer II for inorganic S and P as described.

Each bag contained one and a half tablespoons of moist IRT (2 spoon in the NPK fertilized trial) which yielded a 1 cm thickness when placed in the core with the ring. Moisture contents of the IRT in the flask were approximately 50%, so 7-8 g of dry IRT/ring were used. Total exchange capacity of the soil is 1.46 meq/g (dry basis), thus 10-15 meq/ring were available to trap nutrients from the percolating water. Very rich soil solutions range from 1-2 meq/l O₂, D₂O, and percolated cores, 200%, so each IRT bag was able to handle at least 4 liters of a "rich" solution. For the present study, the amount of microling was well in excess to assure maximum effectiveness in trapping the nutrients percolating through to solution for the 30-day incubation period.

Results and Discussion

Field Isolation

Results from the field isolation are presented in Tables A1.1-A1.3 for the $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and inorganic P .

A higher proportion of $\text{NO}_3\text{-N}$ was trapped in the IIR plots from the clearest area (IIR-02) than in the other areas in the summer season (Table A1.1). In the fall and spring, the 8-year-old site tended to have highest values.

No indication of any treatment effect was observed, except in the IIR plots where about 50% of the total ammonia-N was trapped by the IIR. This suggests that the potential of N leaching out of the surface soil is high.

Very low concentrations of nitrate were observed in all unfertilized sites and seasons (Table A1.2). As a consequence, little nitrate was extracted from the IIR. No effect of treatment was detected, except again in the IIR site where concentrations rose dramatically after the August 1993 fertilization. About 90% of the total $\text{NO}_3\text{-N}$ found at that time was extracted from the IIR. High rates of leaching are expected if this nutrient is not absorbed by vegetation or denitrified, and the high amounts found in the lower resin bag reflects the poor ability of these sandy soils to retain mobile ions.

Inorganic P trapped by the IIR (Table A1.3) ranged from 0 to 10% of the total in the non-fertilized plots. But this value rose to a maximum of 38% when P was added to the sites. Again, these results indicate the low retention capacity of the soil and the high probability of P losses through leaching if conditions are not

Table 21.1. Seasonal $\text{NH}_4\text{-N}$ concentrations (mg/kg) in the soil ($[\text{NH}_4]$), and nitrogen ion exchange fraction ($[\text{N}]_e$), from the various plantation ages and treatments (C = control, B = burned, and PC and BPC fertilized).

ELEMENT (B-PC-BLE)												
SUMMER					FALL				SPRING			
Tb	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}^2	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}
C	n	34.4	8.5	5.9	40.8	8.4	5.5	1.1	34.7	8.0	4.8	0.8
	SB	30.0	4.8		8.4	5.4			3.7	3.4		
B	n	13.8	10.0	1.0	21.1	8.3	3.3	1.0	13.3	3.4	2.4	0.8
	SB	3.4	3.4		2.0	1.0			0.4	0.3		
PC	n	30.0	6.2	4.4	42.4	5.4	4.3	1.3	13.2	4.4	4.3	0.3
	SB	5.1	1.7		1.3	1.3			1.3	1.3		
B-PC-BLE PLANTATION												
SUMMER					FALL				SPRING			
Tb	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}
C	n	15.4	11.4	1.0	8.1	5.0	4.0	1.0	10.8	4.3	3.8	0.5-11.8
	SB	4.1	4.1		1.3	1.3			3.4	2.3		
B	n	10.1	7.5	1.4	18.0	2.4	2.2	0.4	15.4	3.4	1.2	0.2
	SB	0.4	1.9		1.3	1.3			1.4	1.4		
PC	n	30.0	5.3	1.4	14.7	6.7	3.2	1.3	11.9	4.7	3.9	0.1
	SB	5.4	1.1		2.9	1.5			0.9	0.4		
B-PC-BLE PLANTATION												
SUMMER					FALL				SPRING			
Tb	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}	$[\text{NH}_4]$	$[\text{N}]_e$	$[\text{N}]_e$	\bar{e}
C	n	30.0	8.4	0.0	21.3	3.0	3.0	0.0	3.1	2.1	0.0	0.0
	SB	4.4	5.3		0.4	0.4			1.4	1.3		
B	n	20.0	9.2	0.0	1.0	5.1	3.9	0.2	4.9	3.4	2.7	0.1
	SB	0.4	0.4		0.1	0.0			1.1	1.1		
PC	n	30.0	1.7	1.7	18.3	9.2	4.3	0.3	12.3	4.2	4.1	0.1
	SB	3.2	1.3		0.9	0.4			0.7	0.0		
BPC	n	17.4	8.4	0.0	44.7	17.0	13.3	43.7	10.2	18.4	8.4	0.0-12.4
	SB	7.1	2.1		18.3	14.0			4.1	2.0		

$$\bar{e} = [\text{N}]_e - [\text{N}]_e$$

\bar{e} = percent from total trapped in the ion exchange ratio.

Table 41.2. Dissolved $\text{NH}_4\text{-N}$ concentrations (mg/kg) in the soil (0-10, and bottom run exchange) Profile (0-15), from the plantation ages and treatments (C = control, B = burned, and PE and NPE fertilized).

CLIMATE (B-TN-BL)												
SUMMER					FALL				SPRING			
TN		(0-1)	(1-5)	(5-15)	z	(0-1)	(1-5)	(5-15)	z	(0-1)	(1-5)	(5-15)
C	x	0.8	0.8	0.0	0.0	0.6	0.6	0.0	0.0	0.8	0.8	0.0
	SD	0.1	0.2			0.2	0.2			0.2	0.1	
B	x	1.0	0.3	0.1	10.0	0.4	0.3	0.1	10.7	0.8	0.8	0.0
	SD	0.2	0.1			0.2	0.2			0.8	0.8	
PE	x	0.8	0.8	0.0	0.0	0.7	0.7	0.0	0.0	0.7	0.7	0.0
	SD	0.1	0.1			0.1	0.1			0.3	0.3	

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6-16-OLD PLANTATION

SUMMER					FALL				SPRING			
TN		(0-1)	(1-5)	(5-15)	z	(0-1)	(1-5)	(5-15)	z	(0-1)	(1-5)	(5-15)
C	x	1.2	1.2	0.8	0.8	0.7	0.6	0.1	10.3	0.3	0.3	0.0
	SD	0.0	0.0			0.2	0.2			0.8	0.8	
B	x	---	---	---	---	0.4	0.4	0.1	10.3	0.4	0.4	0.0
	SD	---	---			0.1	0.1			0.1	0.1	
PE	x	1.2	1.1	0.1	0.1	0.7	0.5	0.1	10.4	0.3	0.3	0.0
	SD	0.0	0.0			0.2	0.1			0.8	0.8	

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29-36-OLD PLANTATION

SUMMER					FALL				SPRING			
TN		(0-1)	(1-5)	(5-15)	z	(0-1)	(1-5)	(5-15)	z	(0-1)	(1-5)	(5-15)
C	x	1.3	1.0	0.1	9.1	0.4	0.4	0.0	0.0	0.4	0.4	0.0
	SD	0.1	0.1			0.2	0.2			0.8	0.8	
B	x	1.1	1.2	0.1	9.1	0.8	0.8	0.0	0.0	0.3	0.3	0.0
	SD	0.1	0.1			0.2	0.1			0.2	0.8	
PE	x	1.8	1.3	0.8	10.0	0.4	0.4	0.0	0.0	0.4	0.4	0.0
	SD	0.4	0.4			0.2	0.1			0.1	0.1	
NPE	x	1.8	1.3	0.3	14.7	0.4	0.4	0.0	0.0	0.8	0.8	0.0
	SD	0.7	0.7			0.7	0.1			0.1	0.1	

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142 (see notes on Table 41.1)

Table A1.5. Seasonal inorganic P concentrations ($\mu\text{g/kg}$) in the soil (C_{10}), and bottom ion exchange resin (C_{10}), from the various plantation ages and treatments (C = control, B = burned, and PB and BPC fertilized).

OLEAST (9-YR-OLD)													
SUMMER				FALL				SPRING					
TB		C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}
C	x	3.2	4.0	8.4	5.7	7.2	7.7	0.1	4.3	3.9	5.0	0.1	3.4
	SD	3.8	3.6			1.8	0.8			1.8	1.0		
B	x	6.7	6.0	8.7	18.4	2.4	2.5	0.2	3.7	3.4	3.4	0.1	7.8
	SD	3.0	3.0			1.8	0.8			1.1	1.0		
PB	x	21.0	17.7	14.3	68.1	17.8	17.2	10.4	58.9	7.8	8.2	1.4	18.4
	SD	6.4	7.0			4.8	4.4			2.4	1.9		
5-YR-OLD PLANTATION													
SUMMER				FALL				SPRING					
TB		C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}
C	x	3.5	2.4	8.1	4.8	1.4	1.3	0.1	7.1	2.2	2.2	0.8	0.9
	SD	0.4	0.4			0.4	0.4			0.2	0.2		
B	x	4.8	4.6	8.2	4.3	1.8	1.4	0.2	11.1	2.4	2.4	0.8	0.8
	SD	1.8	1.8			0.9	0.4			0.4	0.4		
PB	x	41.8	18.4	27.4	58.7	10.3	8.0	21.3	34.4	12.8	8.4	2.4	18.7
	SD	28.3	8.8			14.2	0.7			2.8	1.4		
25-YR-OLD PLANTATION													
SUMMER				FALL				SPRING					
TB		C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}	C_{10}
C	x	4.8	6.2	6.1	2.8	3.8	2.9	0.0	8.0	2.4	2.4	0.1	4.8
	SD	8.5	8.4			0.8	0.7			0.2	0.2		
B	x	6.7	6.8	6.2	4.3	3.7	3.4	8.1	3.7	2.7	2.4	0.1	3.7
	SD	8.9	8.8			0.8	0.8			0.2	0.1		
PB	x	28.3	12.1	18.2	97.2	68.7	77.4	38.9	68.4	15.8	12.4	0.2	21.6
	SD	24.7	3.7			27.6	11.0			7.1	4.8		
BPC	x	28.8	11.0	8.3	42.8	7.3	7.4	4.8	48.8	3.7	3.7	0.4	16.2
	SD	18.4	8.2			2.4	0.7			1.8	1.4		

182 See notes on Table A1.1.

appropriate for P absorption. A strong drop in concentrations was observed over time after the fertilization which may be related to the high leaching losses in previous months.

Laboratory Incubated Cores

Results from the laboratory incubated cores are shown in Tables A1.6-A1.8. In general, between 30-70% of the total NO_3^- -N extracted was found in the drained water regardless of plantation age, season, or treatment, except in the 8PK site in the Fall. In this case, about 70% of the NO_3^- -N was found in the solution (Table A1.6).

Nitrate values were low, as expected (Table A1.6). Nitrification does not proceed under anaerobic conditions, and nitrate present before the incubation would likely be lost through denitrification when moisture contents were increased. The potential denitrification trend was especially evident at the 8PK site where a majority of the initially high Fall nitrate concentrations disappeared after the 30-day incubation.

Inorganic P was also detected in the drained solution from the saturated laboratory cores (Table A1.6). Values usually ranged from 5-10% of the total amounts detected, although some exceptions were observed. Higher concentrations were detected in the solution drained from cores collected in the P fertilized area accounting for 20 to 30% of the total P found.

In general, no special trends were determined when seasons and plantation ages were compared, although field incubated cores tended to have higher concentrations in the DZ than the saturated cores. This suggests that in the field, the soluble P fraction was washed out

Table 81-8. Seasonal $\text{NH}_4\text{-N}$ concentrations ($\mu\text{g/l}$) in the water $\text{C}(\text{NH}_4)_x$ and dechlorinated excess water $\text{C}(\text{NH}_4)_y$, from the various plantation ages and treatments (C = control, B = banded, and PG and SPG fertilized)

CLEARCUT (0-10-BL0)													
SUMMER					FALL					SPRING			
TR	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}^b	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	\bar{x}
C	x	14.5	13.5	3.0	8.0	8.3	8.8	8.7	7.8	8.0	8.8	8.8	8.4
	SD	2.8	2.4			4.3	4.1			2.8	2.1		
B	x	14.4	16.5	3.3	7.7	14.7	13.8	1.8	17.8	18.7	18.8	8.7	8.8
	SD	8.0	8.1			7.8	8.7			8.8	3.8		
PG	x	13.4	12.4	3.4	18.1	15.9	12.5	1.4	10.1	10.2	8.8	8.4	8.8
	SD	3.8	3.8			8.8	4.2			3.3	2.9		
5-10-BL0 PLANTATION													
SUMMER					FALL					SPRING			
TR	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	\bar{x}
C	x	18.8	14.8	8.8	8.8	11.7	9.1	8.2	21.5	12.4	12.4	1.8	8.1
	SD	8.1	7.4			3.8	2.8			7.8	3.4		
B	x	10.7	9.8	1.8	11.8	18.2	10.8	2.4	18.2	12.3	12.8	8.3	8.1
	SD	5.2	4.2			10.8	8.7			8.8	3.8		
PG	x	14.8	12.8	1.8	18.2	14.2	12.8	2.1	14.8	18.4	12.8	1.8	8.7
	SD	3.8	3.8			4.8	4.8			8.7	4.8		
20-10-BL0 PLANTATION													
SUMMER					FALL					SPRING			
TR	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	$\text{C}(\text{NH}_4)_x$	$\text{C}(\text{NH}_4)_y$	$\text{C}(\text{NH}_4)_z$	\bar{x}	\bar{x}
C	x	8.8	7.2	1.1	12.8	21.8	9.8	2.7	14.8	18.8	8.8	2.2	20.8
	SD	8.4	8.8			4.8	4.8			8.4	1.8		
B	x	7.1	7.1	0.8	8.8	11.8	10.8	1.0	8.8	12.8	9.8	1.1	10.1
	SD	3.3	3.3			4.8	4.8			2.8	1.8		
PG	x	18.3	8.8	1.3	12.8	21.8	12.7	3.8	18.1	12.1	10.8	8.8	8.4
	SD	3.8	2.2			7.8	8.8			2.8	2.8		
SPG	x	15.3	18.8	2.7	14.8	21.8	18.8	12.8	18.8	18.8	14.8	1.1	8.8
	SD	7.3	4.2			14.8	10.1			5.8	8.8		

$$\frac{1}{2}[\text{C}(\text{NH}_4)_x + \text{C}(\text{NH}_4)_y]$$

\bar{x} = percent of total found in the dechlorinated water.

Table A1.5. Seasonal NO₃-N concentrations (mg/kg) in the soil (0-10 cm), and drained excess water (g/kg), from the various plantation ages and treatments (C = control, B = burned, and PB and BPA fertilized)

CLEARCUT (0-10-0-0)												
SUMMER				FALL				SPRING				
Tb		[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]
C	x	1.0	1.0	0.0	0.0	0.0	0.1	10.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0		0.0	0.0		0.0	0.0			
B	x	0.0	0.0	0.1	10.0	0.0	0.0	0.0	0.0	0.0	0.1	10.0
	SD	0.1	0.1		0.0	0.0		0.1	0.0			
PB	x	0.0	0.0	0.1	10.0	0.0	0.0	0.1	10.0	0.0	0.0	10.0
	SD	0.1	0.1		0.0	0.0		0.1	0.0			
0-10-0-0 PLANTATION												
SUMMER				FALL				SPRING				
Tb		[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]
C	x	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0
	SD	0.1	0.1		0.0	0.0		0.0	0.0			
B	x	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0		0.0	0.0		0.1	0.1			
PB	x	0.0	0.0	0.1	10.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.1		0.0	0.0		0.0	0.0			
0-10-0-0 PLANTATION												
SUMMER				FALL				SPRING				
Tb		[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]	[N]
C	x	0.0	0.0	0.1	10.0	0.0	0.0	10.0	0.0	0.0	0.0	10.0
	SD	0.1	0.0		0.0	0.0		0.1	0.0			
B	x	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	10.0
	SD	0.1	0.1		0.0	0.0		0.1	0.0			
PB	x	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.1	0.1		0.0	0.0		0.1	0.1			
BPA	x	0.0	0.0	0.0	0.0	0.0	1.0	10.0	0.0	0.0	0.0	10.0
	SD	0.1	0.1		0.0	0.0		0.1	0.1			

SD = values are Table A1.4

Table A1.5. Seasonal inorganic P concentrations (mg/kg) in the soil (0/10), and drained excess water (1/0), from the various ploughing ages and treatments (C = control, B = burned, and P0 and P60 Fertilized).

0/10-0/10													
SUMMER													
T0		C0 ¹	C10	C60	s ²	C0 ¹	C10	C60	s	C0 ¹	C10	C60	s
C	x	4.0	4.2	0.0	3.1	2.0	2.1	0.4	14.0	4.4	4.1	0.1	4.2
	SD	1.1	1.2			1.1	0.4			2.2	0.1		
B	x	10.0	9.1	0.0	8.0	3.0	3.1	0.4	11.4	1.7	1.2	0.0	10.4
	SD	3.2	4.4			0.7	0.6			0.7	0.0		
P0	x	10.0	10.2	1.4	11.3	10.0	9.4	0.0	27.1	8.7	7.0	1.1	10.4
	SD	5.1	1.0			7.2	4.8			3.5	1.0		
10-10-0/10 PLANTATION													
SUMMER													
T0		C0 ¹	C10	C60	s	C0 ¹	C10	C60	s	C0 ¹	C10	C60	s
C	x	4.0	1.1	0.1	4.0	0.0	1.0	0.1	10.0	1.5	1.1	0.4	10.0
	SD	1.7	1.1			1.0	0.0			0.5	0.0		
B	x	4.5	0.1	0.1	3.4	0.0	0.0	0.4	10.1	4.0	1.0	0.4	10.1
	SD	3.4	1.0			1.3	0.4			1.0	1.0		
P0	x	10.1	11.1	1.0	10.0	10.1	10.1	0.1	40.1	10.1	14.1	4.1	17.1
	SD	17.0	14.0			9.0	0.0			13.0	0.1		
10-10-0/10 PLANTATION													
SUMMER													
T0		C0 ¹	C10	C60	s	C0 ¹	C10	C60	s	C0 ¹	C10	C60	s
C	x	4.4	4.2	0.1	4.0	0.4	0.1	0.1	4.2	1.7	1.4	0.0	10.4
	SD	0.1	0.1			0.0	0.0			0.0	0.0		
B	x	0.1	0.1	0.1	1.0	0.4	0.0	0.1	10.4	0.1	1.0	0.0	10.1
	SD	1.0	1.0			0.4	1.4			0.0	0.1		
P0	x	10.1	11.1	0.1	10.1	10.4	10.0	10.0	10.1	10.1	10.4	0.0	10.1
	SD	10.0	10.1			10.1	10.1			0.1	0.1		
P60	x	10.0	0.1	0.4	10.0	0.0	0.0	1.0	40.0	1.0	1.4	0.1	4.1
	SD	4.4	1.0			1.4	1.0			0.0	0.0		

¹See notes on Table A1.4.

by percolating water, providing a possible concentration equilibration between the soil particles and the liquid phase.

Conclusions

- (1) Relatively low amounts of K and P were detected in the lower IR bags (field cores) or the drained solution (saturated laboratory cores) for the non-fertilized plots when compared with the fertilized ones.
- (2) Leaching potential losses in the field are high, especially in the fertilized plots and the drainage sites.
- (3) Field cores showed that amounts of leachable ions are higher than in the saturated laboratory cores where solution does not move.
- (4) Amounts of K and P detected in the lower IR's or drained water was related to the amount mineralized.

APPENDIX 2

SURFACE SOIL VARIABLES FOR THE NON-SYMBIOTIC N FIXATION CORPS

Table A2.1. Seasons (S = summer, F = fall, and Sp = spring) average concentrations and standard deviations (mg/kg, except where specified) for various soil surface characteristics for the different plantation ages and treatments in the non-symbiotic biological nitrogen fixation soil cores. AB = molist alkyline production/core-yr (ammonium reduction, net log. transform, see Methods); CPE = cationic activity; TN = total nitrogen; ACS = cationic content.

	CLARKSON SITE (8-yr-old)								
	Control			Summer			PB-fertilized		
	S	F	Sp	S	F	Sp	S	F	Sp
AB	238.0	187.1	267.4	305.8	54.3	288.0	93.7	45.0	90.3
	187.0	87.1	181.0	111.1	38.8	436.3	62.0	51.4	39.6
AI	128.8	128.4	88.0	88.4	91.8	17.4	71.2	108.4	82.8
	48.8	83.4	88.2	36.2	44.8	85.8	25.4	74.8	95.4
Ca	88.4	88.8	88.8	88.8	88.8	88.8	88.8	188.8	88.8
	47.8	88.8	88.8	88.2	88.2	88.4	88.4	188.8	88.8
Fe	---	88.4	28.2	---	28.0	28.8	---	28.2	28.8
	---	88.8	88.2	---	28.1	28.2	---	28.8	18.8
N	11.2	11.2	8.8	18.8	8.8	8.8	18.8	17.4	18.8
	4.4	4.2	1.4	3.4	8.8	3.4	8.2	8.1	8.8
Mg	28.0	28.4	14.4	18.8	18.2	18.8	14.4	28.4	18.2
	18.2	28.8	28.8	7.2	3.8	8.4	8.2	28.2	2.2
Mn	8.8	8.4	8.2	8.8	8.8	8.8	8.4	8.2	8.4
	8.2	8.2	8.1	8.2	8.8	8.2	8.2	8.2	8.2
CPE	2.8	1.4	1.4	1.8	1.4	1.8	1.8	1.8	1.4
	1.8	8.8	8.8	8.2	8.8	8.2	8.8	8.8	8.4
PH	4.2	4.1	4.2	4.8	4.8	4.2	4.8	4.8	4.8
	8.2	8.2	8.2	8.8	8.1	8.2	8.8	8.2	8.8
P	2.2	1.8	8.2	2.2	1.2	2.2	4.8	8.8	8.8
	8.8	8.8	8.8	8.2	8.4	8.8	2.2	8.2	2.2
TN	488.0	488.0	188.8	188.8	188.8	188.8	488.8	527.2	188.8
	188.8	28.8	187.2	18.8	188.8	18.8	87.4	288.4	84.8
ACS	18.1	18.8	18.8	18.8	18.8	18.8	18.1	18.8	18.8
	8.8	3.8	3.8	8.4	8.8	8.8	8.8	8.8	18.8
Zn	---	8.8	8.4	---	1.8	1.8	---	8.8	8.8
	---	8.8	8.2	---	1.2	1.2	---	8.2	8.8

Table A6.2., continued.

	9-16-8-0 PLANTATION								
	Control			Burned			F0-fertilized		
	S	F	SP	S	F	SP	S	F	SP
Alt	66.1	52.2	73.4	126.3	47.4	126.3	55.2	163.6	761.1
	25.1	26.8	46.4	100.0	39.8	100.0	116.8	206.1	146.2
Al	75.2	106.0	102.4	47.7	92.8	34.8	56.2	56.4	46.4
	105.3	175.8	121.7	39.5	40.3	12.4	41.3	26.3	10.5
Ca	76.0	127.3	72.0	100.0	106.4	100.0	110.0	71.3	71.4
	42.3	126.5	44.4	44.1	34.3	55.7	46.4	23.7	23.9
Fe	—	21.0	57.9	—	21.7	13.3	—	33.2	29.8
	—	24.7	26.7	—	14.3	4.8	—	26.3	23.4
K	6.8	11.2	9.4	11.2	12.8	6.4	13.4	13.4	14.4
	2.8	7.3	3.7	1.8	4.8	2.3	8.3	4.4	23.4
Mg	14.8	16.4	15.2	12.4	17.2	17.4	20.8	13.4	15.2
	11.0	16.4	9.1	9.4	17.3	6.7	9.1	8.4	6.7
Mn	0.5	0.4	0.3	0.7	0.4	0.9	0.7	0.4	0.4
	0.3	0.1	0.1	0.3	0.4	1.1	0.7	0.4	0.7
OM	2.7	1.4	1.8	1.8	1.4	1.4	1.3	1.4	1.2
	0.7	1.4	0.5	1.8	1.3	0.4	1.0	1.4	0.7
pH	4.7	4.1	4.2	4.4	4.1	4.1	4.4	3.9	3.9
	5.1	5.1	4.2	5.1	5.1	5.2	5.1	5.2	5.2
P	1.7	2.0	1.7	2.2	2.2	2.0	2.7	2.0	4.0
	0.4	0.2	0.3	0.4	0.4	0.4	2.4	3.1	0.9
TN	426.8	553.4	523.2	600.4	580.8	594.2	421.2	528.4	530.4
	126.4	126.4	160.2	166.8	219.4	126.1	126.1	214.7	123.7
WC	14.4	25.2	24.4	15.1	15.3	15.4	14.4	25.3	24.2
	4.4	5.3	5.1	1.3	4.8	2.4	1.4	4.4	5.1
Zn	—	5.3	0.7	—	1.3	1.0	—	4.4	1.7
	—	1.0	0.1	—	1.8	0.4	—	0.1	1.2

Table A3.3. - continued

	26-28-30.3 PLANTATION								
	Control			Burned			PG-4ert571-ized		
	S	F	SP	S	F	SP	S	F	SP
AB	46.0	28.8	236.1	38.1	38.4	208.0	58.4	26.8	439.0
	18.3	22.1	234.3	40.4	62.8	50.3	48.7	38.7	234.3
AL	67.2	69.8	97.8	66.8	55.2	68.8	61.8	28.8	48.8
	48.8	48.2	20.8	50.7	31.8	35.4	38.1	38.2	33.4
Ca	32.0	33.0	33.8	28.8	28.4	28.4	112.0	61.8	80.8
	13.3	23.8	26.8	13.8	8.3	28.8	148.8	48.7	26.8
Fe	—	48.8	35.8	—	38.3	21.8	—	48.8	23.1
	—	21.0	11.2	—	12.8	18.8	—	23.7	13.8
C	8.4	8.8	8.8	8.8	8.8	8.8	28.2	21.8	12.8
	2.2	0.1	2.2	1.8	2.2	2.2	12.1	8.3	1.8
Mg	22.8	13.8	11.2	12.8	18.4	28.8	28.8	23.2	12.8
	8.2	8.4	8.8	8.2	2.2	8.1	33.8	27.8	2.3
Nu	0.2	0.3	0.2	0.2	0.8	0.1	0.4	0.8	0.3
	8.1	8.3	0.3	0.4	0.2	0.0	8.3	0.8	0.2
NO3	1.1	1.2	1.8	2.3	0.8	1.8	1.3	1.8	1.8
	0.2	0.8	0.8	0.8	0.8	0.8	2.7	1.2	0.2
pH	4.8	4.8	4.8	4.8	4.0	4.8	4.4	5.2	3.8
	0.3	0.1	0.2	0.3	0.1	0.1	0.8	0.8	0.3
P	2.1	1.8	1.8	2.0	1.2	2.0	6.8	11.8	8.8
	0.8	0.8	0.8	0.2	0.8	0.8	2.8	8.8	8.8
TM	677.0	417.2	388.8	677.0	628.2	294.8	707.4	448.8	418.4
	288.1	118.8	184.8	118.1	158.8	81.7	234.4	218.7	87.8
WCI	18.8	18.8	18.8	18.8	18.8	18.0	18.8	18.8	18.8
	1.8	3.7	4.2	1.2	4.4	1.4	3.8	4.3	4.3
Zn	—	0.8	0.2	—	0.8	0.8	—	0.8	0.8
	—	0.3	0.3	—	0.8	0.8	—	0.3	0.3

Table A3.1., continued

19-19-0-0 PLANTATION			
NPK FERTILIZER			
	SUMMER	FALL	SPRING
N	26.8	8.5	44.9
	7.4	8.8	24.9
P	22.4	29.8	26.6
	8.8	8.2	8.7
K	26.2	21.2	22.8
	15.4	17.2	28.4
Fe	---	12.1	8.1
	---	8.4	6.9
E	27.8	28.4	24.4
	26.9	14.4	22.7
Mg	8.8	8.8	7.2
	2.2	4.8	1.8
Mn	6.2	8.4	6.2
	6.8	8.2	6.1
MO	1.4	2.4	1.2
	6.2	8.2	6.2
Ca	4.8	4.2	4.8
	4.5	8.2	6.1
P	8.4	2.8	1.8
	4.8	2.4	6.8
Na	222.8	242.4	262.2
	28.8	48.4	48.4
NO ₃	34.2	28.2	24.2
	2.2	8.8	1.1
Cl	---	7.2	1.2
	---	8.8	1.1

APPENDIX 4

LABORATORY P RESULTS

Introduction

In this section, P concentrations changes after the 30-day incubation period are presented. This study was performed simultaneously with the S mineralization investigation to determine if there was relationship between the two processes related Oola and Reil, 1981).

Double acid extractable soil P concentrations were measured before and after a 30-day incubation period in the field and laboratory. The lower ion exchange resin (extracted with KCl) and the deionized water from the field and saturated laboratory sites, respectively, were included in the analysis. Details of the procedures used are described in the Methods section in the dissertation text, and in Appendix 1.

Results

During the summer, an interaction between plantation age and treatment was found ($p = 0.04$, Tables 34-1-34-2). When plantation ages were compared within treatments, no differences were detected in the control and burned areas, although they tended to have slightly higher concentrations in the clearcut area than in the other areas. On the other hand, concentrations of P in the PG plots were at least 1.5 times as large in the 8- and 20-year-old stands ($p = 0.03$) than those found in the clearcut area (Table 34-1.)

Table A6.3. Liver cell inorganic P concentrations ($\mu\text{g P/g}$) for the various treatments and plantages. Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	PLANTING AGES (cm)		
	0	8	12
Control	5.8 a	3.2 a	4.2 a
Burned	4.9 a	3.8 a	5.0 a
PE	25.2 b	23.8 b	25.0 b

Table A6.2. Sower soil P concentrations changes ($\mu\text{g P/kg-soil}$) for the various treatments and phosphorus. Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	PLANTING AGES [yrs]		
	0	5	20
Control	1.2 a	0.8 a	1.1 a
Buried	1.8 a	1.7 a	0.8 a
PK	-1.1 a	-4.7 b	2.0 a

As expected, when treatments were compared within plantation age, in all cases PC plots had significantly higher ($p < 0.05$) concentrations which were at least 3 times larger than the control or burned plot levels.

When changes in P concentrations were determined before and after the incubation period, the burned plots had at least 1.7 times higher concentrations than the control plots in the 15-year and 20-year-old plantation (Table A6.2), and the PC plots had at least 2.7 higher values in the 0- and 20-year-old stands. Negative values were observed in the PC plots from the 15-year area. No treatment values were statistically different from the controls except in the PC treated plots in the 0-year-old plantation.

During the fall, interactions between treatment and incubation method and between treatment and plantation age, were significant at the 0.0001 level.

When treatments were compared within incubation methods (Table A6.3), control and burned plots showed no significant differences in P concentrations, and changes after the incubation period were near 0 or negative (Table A6.4). On the other hand, P concentrations in the PC plots were at least 5 times larger than the other treatments, and relatively high increases in concentration after the incubation period were found in these plots; they were significantly different ($p < 0.05$) in the field and the saturated incubation when compared with the same incubation methods in the control plots. A relatively large portion of the P detected in the field incubated cores came from the lower ion exchange resins (see Appendix 1).

Table A4.1. Kd with inorganic P concentrations (mg P/kg) for the various treatments and incubation methods (IE = initial incubation, F = Field, H = unamended laboratory, and L = amended laboratory cases). Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	INCUBATION METHOD			
	IE	F	H	L
Control	1.3 a	1.3 a	1.3 a	1.4 a
Amended	1.4 a	1.3 a	1.3 a	1.4 a
PC	24.1 b	28.8 b	25.8 b	22.8 b

Table 34.1. Fall wet P concentration changes (mg P/kgsoil) for the various treatments and incubation methods (IC = initial conditions, F = field, L = simulated laboratory, and S = saturated laboratory cores). Different letters within a column indicate significant differences ($P = 0.05$) from controls.

Treatment	INCUBATION METHOD		
	IC	L	S
Control	-0.4 a	-0.3 a	0.1 a
Revised	-0.1 a	0.3 a	1.8 a
PC	13.7 b	1.2 a	3.7 b

When incubation methods were compared within treatments (Table A6.2) no effects were detected in the control and burned plots. On the other hand, the field and saturated cores from the PC fertilized plots tended to have higher concentrations of P than initial conditions or unsaturated cores, but only the field cores were significantly different ($p = 0.05$) from the initial conditions cores.

Plantation age also had some effect on inorganic P concentrations, but only in those plots fertilized with P_i (Table A6.3). Significantly higher ($p = 0.05$) concentrations were detected in the oldest stand and in the 8-year-old plantation when compared with the clearest site. The 28-year-old stand also had significantly higher P levels than the 8-year-old site ($p = 0.005$).

When changes in P concentrations after the incubation period were investigated (Table A6.4), clearest values tended to be negative, even in the PC fertilized plots. In the other areas values tended to increase and were especially high in the PC plots.

During the spring, the plantation age-treatment interaction was statistically significant ($p = 0.002$, Tables A6.5-A6.8). As in the fall, control and burned plots had smaller P concentrations when they were compared among the different areas. But in the PC fertilized plots, the clearest site had approximately half the concentrations observed in the other two areas ($p = 0.05$). No differences were observed between the 8-year-old and the 28-year-old plantations.

When changes in P concentrations after the incubation period were studied, in a majority of the cases values were negative or near 0 (Table A6.9). There was a decrease in P concentrations in the PC fertilized plots of the 8-year-old plantation, contrary to the results

Table 24.6. Fall soil inorganic P concentrations (mg/kg) for the various plantations ages and treatments. Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	PLANTATION AGE (yrs)		
	0	5	20
Control	2.3 a	2.6 a	2.2 a
Burned	2.9 a	2.8 a	2.6 a
P0	23.1 b	22.4 b	46.3 b

Table 10.1. Fe(II) and P concentrations changes (mg P/kg-soil) for the various plantation ages and treatments. Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	PLANTATION AGE (yrs)		
	0	5	10
Control	-0.1 a	-0.2 a	-0.4 a
Burned	0.7 a	0.5 a	0.9 a
PK	-0.4 a	7.3 b	13.5 b

Table 86.7. Spring soil inorganic P concentrations (mg/kg) for the various plantation ages and treatments. Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	PLANTATION AGE (Yrs)		
	0	8	29
Control	3.3 a	3.3 a	3.1 a
Forest	3.3 a	3.3 a	3.3 a
FE	8.0 b	16.2 b	27.4 b

Table H4.6—Spring net P concentration changes (mg P/kg-m) for the various plantation ages and treatments. Different letters within a column indicate significant differences ($p < 0.05$) from controls.

Treatment	PLANTATION AGE (yrs)		
	0	8	25
Control	0.8 a	0.0 a	-0.2 a
Burned	-0.2 a	-0.4 a	-0.2 a
Pt	-0.7 a	-1.4 b	2.2 b

observed in the 20-year-old stand. In this later site, almost 3 mg P/kg-so were produced.

In the following sections, results comparing the different seasons within each of the three sites will be presented.

In the clearcut area, a 3-way interaction between season, treatment, and incubation method was significant ($p = 0.04$, Tables 84, 9-85, 10). When seasons were studied within each of the incubation methods and treatments, inorganic P concentrations decreased in the fall and spring to about half the summer values. In spring, concentrations tended to increase somewhat from fall levels.

The above tendencies were higher in the PE fertilized plots as a result of the early October fertilization. P concentrations in the fall were statistically similar to those analyzed in the summer, although much higher concentrations were expected after the application of fertilizer in the same year. A strong decrease, to about half of the fall concentrations, was observed in spring.

When changes in P concentration after the incubation period (Table 84, 10), a decrease in concentrations at the end of the incubation period was observed in the fall and spring, especially in the selected cores from the burned plots (these were statistically different, $p = 0.05$, when compared to control rates). In the PE fertilized treatment, negative values were observed in the summer (especially in the unselected cores). In the fall, the field incubated cores showed a statistically significant ($p = 0.05$) increase in amounts after the incubation period (approx. 4.8 mg P/kg-so) when compared with summer or spring rates. In the same period, the

Table 24.6 Seasonal soil inorganic P concentrations ($\mu\text{g P/kg}$) in the observed area for the various incubation methods (IC = initial) conditions, F = field, U = unamended laboratory, and E = amended laboratory areas) and treatments. Different letters within a column indicate significant differences (prob. level 0.05) from summer.

CONTROL INCUBATION METHOD				
Season	IC	F	U	E
Summer	6.2 a	5.2 a	6.5 a	6.1 a
Fall	5.4 a	2.3 a	2.1 b	2.0 b
Spring	5.8 a	2.9 a	3.0 a	6.4 a
FERTILIZED INCUBATION METHOD				
Season	IC	F	U	E
Summer	6.2 a	6.7 a	6.2 a	10.0 a
Fall	2.6 a	2.6 b	2.3 a	2.6 b
Spring	3.7 a	2.6 a	2.2 a	2.3 b
FERTILIZED INCUBATION METHOD				
Season	IC	F	U	E
Summer	21.6 a	20.0 a	17.6 a	22.8 a
Fall	24.8 a	19.0 a	16.6 b	22.0 b
Spring	9.5 b	7.6 b	7.2 b	8.7 b

Table 84-15. Seasonal net P concentration changes (mg dry wt⁻¹) in the cleared area for the various incubation methods (F = field, U = unsaturated laboratory, and S = saturated laboratory cores) treatments. Different letters within a column indicate significant differences (p < 0.05) from summer.

CONTROL INCUBATION METHOD			
Season	F	U	S
Summer	1.0 a	2.1 a	2.8 a
Fall	-0.2 a	-0.3 a	0.3 a
Spring	-0.1 a	-0.6 a	1.4 a
BURNED INCUBATION METHOD			
Season	F	U	S
Summer	1.9 a	1.4 a	6.8 a
Fall	8.2 a	0.3 a	1.1 b
Spring	-0.1 a	-0.8 a	0.8 b
PK FERTILIZED INCUBATION METHOD			
Season	F	U	S
Summer	-0.8 a	-0.8 a	1.2 a
Fall	2.1 b	-0.2 a	-0.0 b
Spring	-0.3 a	-1.3 a	0.8 a

unfertilized and unfertilized sites had high negative values which were significant ($p = 0.000$) in the unfertilized sites when compared with the summer and spring rates.

In the 10-year-old plantation, a highly significant ($p = 0.000$) interaction between treatment and season was observed (Tables A4.11-A4.12). When seasons were compared within each of the treatments, no statistical differences were detected in the control or burned plots, although P concentrations tended to drop in the fall and spring (Table A4.11). PG fertilized plots showed a decrease in concentrations (more than 50% over the year) with fall and spring values significantly lower from summer values ($p = 0.00$).

When changes in P concentrations after the incubation period were calculated (Table A4.12), a relatively strong increase was observed in the PG plots in the summer and fall, but drastically dropped in the spring ($p = 0.00$), from 7.2 mg P/kg-mg to -4.4 mg P/kg-mg. Rates in the control and burned plots, although non-statistically differing from summer values, also tended to decrease with season to negative values in spring. In the 30-year-old plantation, the treatment-season interaction was also significant ($p = 0.002$). Average P concentrations and rates are shown in Tables A4.13 and A4.14.

When the different seasons were compared, statistically similar P concentrations were found for the control and burned plots, although they tended to drop by half in the fall and spring from summer values (Table A4.12). In the other hand, in the PG fertilized plots, concentrations almost doubled in the fall ($p = 0.00$) when compared with summer concentrations following the second application of PG fertilizer in October, but decreased 2.4 times in the spring from fall levels ($p = 0.00$).

Table A4.21. Seasonal soil organic P concentrations (mg P/kg) in the Norway spruce plantation for the various treatments. Different letters within a column indicate significant differences ($p < 0.05$) from Summer.

Season	TREATMENT		
	Control	Burned	P0 fertilized
Summer	3.2 a	3.0 a	23.9 a
Fall	3.3 a	3.0 a	22.4 a
Spring	3.1 a	3.0 a	20.4 a

Table A6.12. Seasonal net P concentration changes (mg P/kg dry) in the 8-yr-old plantation for the various treatments. Different letters within a column indicate significant differences ($p < 0.05$) from summer.

Season	Fertilizer		
	Control	Banded	nt fertilized
Summer	0.0 a	1.2 a	4.2 a
Fall	0.2 a	-0.5 a	2.3 a
Spring	0.0 a	-0.4 a	-0.6 b

Table A6.23. Seasonal soil inorganic P concentrations (mg P/kg) in the 20-yr-old plantation for the various treatments. Different letters within a column indicate significant differences ($p < 0.05$) from summer.

Season	TREATMENT			
	Control	Burned	80 Fertilized	900 Fertilized
Summer	4.2 a	6.0 a	75.0 a	12.7 a
Fall	3.0 a	3.4 a	88.3 b	5.3 b
Spring	2.1 a	2.3 a	17.4 b	3.1 b

Table 44.14. Seasonal net P concentration changes (mg P/kg-so) in the 25-yr-old plantation for the various treatments. Different letters within a column indicate significant differences ($p < 0.05$) from summer.

Season	TREATMENT			
	Control	Normal	PK fertilized	SPK fertilized
Summer	1.1 a	0.6 a	1.8 a	4.2 a
Fall	-0.4 a	0.1 a	11.3 b	0.1 b
Spring	-0.3 a	-0.2 a	3.7 a	-3.6 b

The P concentration after the incubation period increased in the PE fertilization in the fall after the second application of P (Table A6.14). Rates quadrupled in the fall but decreased again during the spring incubation to concentrations similar to those found in the summer. In control and burned plots, changes in P concentrations after the incubation period were small over the summer and, in general, they were less or even negative, especially in the spring (Table A6.14).

When the NPK treatment was added in the statistical analysis of P concentration changes over the incubation period for the 20-year-old plantation, the following results were obtained (see Tables A6.15-A6.16):

- 1) When comparing the treatments within the seasons, the NPK fertilized plots had lower inorganic P concentrations than the PE plots ($p = 0.05$), but were at least 2 times higher than the control and burned plots in the summer and fall seasons (although only in the summer they were statistically different from control at $p = 0.05$). In the spring, the NPK treated sites had similar concentrations as the control sites.
- 2) A significant increase (about 2-4 times) in P concentrations was observed in the fall and spring seasons ($p = 0.05$) when compared with the summer. Although the site was fertilized with more P in August 1983, only less than 4 months before the fall sampling was conducted, no increase in P concentration was observed.
- 3) When changes in P concentration after the incubation period were studied in the summer, the NPK plots had higher concentration changes than the PE (4.2 mg P/kg-so in the NPK, vs 2.8 mg P/kg-so

in the fall, $p = 0.05$). But in the fall and spring, concentrations changed considerably to near 0 or less, in the spring (Table 44.14). The net change in concentrations after the incubation period in the PK plots were 33 times and 6 times larger in the fall and spring, respectively, when compared with the BK plots.

Discussion

Low availability of P (as well as of K) is a major factor regulating plant distribution and productivity of both natural and managed ecosystems in Florida. For example, moderate to large volume gains have been observed in southern slash pine plantations after fertilization with P and often without K (Kaufman et al., 1973; Fisher and Larkert, 1980), depending on the soil characteristics of the site.

In addition to possible direct effects on plant nutrient uptake and growth, addition of fertilizers may change activity of microbial processes in the soil. Adequate availability of P has been shown to be an important factor controlling nitrification, nitrification, and biological H_2 fixation (Dale and Weil, 1981) thus contributing to the proper functioning of the entire ecosystem.

In highly weathered sandy soils, like those found in southern Florida, availability of P is largely controlled by the release of inorganic P from organically bound P through the microbial mineralization process (Morrison, 1982). Once mineralized, inorganic P may be leached, absorbed by the vegetation or immobilized in microbial biomass, become bound to the cation exchange sites of the mineral soil or organic matter, or may be complexed with Al or Fe under acid conditions.

In the present investigation, different results were obtained depending on treatment, plantation age, and season. Invasive species had little effect except during the fall.

During the summer (Table A4.2-A4.5), the slightly higher P concentrations in the non-P fertilized clearcut plots when compared to the other two areas, may have been a result of the recent clearcutting. In the other hand, the 3-year-old plantation had the lowest initial P concentrations with no changes in P concentration at the end of the incubation period similar to those found in the oldest stand. These low initial concentrations may have been related to the more active uptake of this nutrient by the vegetation of this site (Gholz, Fisher, and Fitzhugh, in press).

When PE treated plots were compared among plantation ages and other treatments, different tendencies were observed. Net changes in P concentration after the incubation period in the clearcut site were at least 3 mg P/kg-mg lower than those in the other treatments in the same plot, and at least 4 mg P/kg-mg lower than in other plantations. It is difficult to explain why these results occurred, except to speculate that the increases in P due to the recent disturbance in the clearcut area were not sufficient to stimulate microbial decomposition, but when additional P was added to the area through fertilization, microbial activity was effectively increased. This increased availability of P in the PE fertilized plots may have had same effect. Similar tendencies were observed in the fall and spring (Table A4.3-A4.5, A4.7-A4.9). Again, the control and burned plots in the clearcut site had higher average concentrations and they tended to increase during the incubation period when compared with the other two plantations, but

differences were less striking. In the PE treated plots, AgI concentrations and rates were half or less, respectively, in the clearcut area than in the other sites. Spring concentrations were also about 2 times less, but net changes in P concentrations were lower only when compared with the 20-year-old site.

Burned plots showed slightly higher concentrations before and after the incubation period than control plots in all areas. Highest values were obtained in the summer, 2 weeks after the fire. This indicates that burning released P, although effects seem to be temporary and small. Regular burning of the site, then, may facilitate and increase availability of P for vegetation growth, and would accelerate P release from the litter layer. In the clearcut site, however, burning may be potentially detrimental as little vegetation was present during the first year to take advantage of any increase, as P losses through leaching are expected to increase. Analysis of the litter for exchange cations (Appendix 1) indicates that the amount of P trapped by the resin increases in the burned plots but only represented about 10% of the total extractable P detected for the cores.

Phosphorus fertilization increased extractable P in all seasons, as expected, but effects on P concentration changes after the incubation period varied among locations and seasons. In general, P concentrations increased in the 5- and 20-year-old plantations during the summer and fall, but decreased in the clearcut in all seasons and in the 8-year-old plantation in the spring, after the cores were incubated.

Finally, when concentrations were studied among seasons, a strong decline was observed in the fall and spring (Table 36.3-36.14).

Negative values in the spring after the 30-day incubation may have been the product of microbial growth increases during this period of "bad weather". Also, large decreases in concentrations were found in the P fertilized plots, especially in the clearest site and in the spring. In the NP site, levels dropped almost to control concentrations in the fall, about 4 months after the latest P addition. These results suggest that, either very high uptake rates were occurring, or that P losses through complexation with Al and Fe, or leaching were high. Losses would be expected to be higher in the disturbed area, as little or no vegetation was present, and more water would percolate through the surface soil. Results from the lower ion exchange resin analysis indicates that 50 to 75% of the total extractable P in the P fertilized plots were extracted from the resin. This suggests that the potential for leaching losses is very high in fertilized sites (Appendix I).

No remarkable tendencies were observed among incubation methods except in the fall (Table A1-D-A1, A2). Higher concentrations occurred in the field and saturated incubated cores. As mentioned, in the P fertilized plots, relatively large quantities of P were extracted from the ion exchange resin [see Appendix I], contributing to the elevated values in the cores from this treatment. This may have been a result of drying and wetting cycles releasing labile organic P, or to increased P solubility after several soil solution washes through the cores. The increase of P in the saturated cores may also have been due to an increase in the solubility of P from desorption-desorption sites, in addition to mineralization processes (Boddy and Lee, 1980). Also, Boddy and Patrick (1982) indicated that such an increase in P

availability under anamorphic conditions may be due to the reduction of certain inorganic forms of P, especially Fe-P forms, and to an increase solubility of organic matter which helps release P.

In the majority of the cases, P concentrations before and after the incubation period were not significantly different when incubation methods were compared within treatment, suggesting that other factors besides temperature, initial inorganic P concentrations, and soil solution contact were regulating P processes. No significant increases in extractable P were detected in the laboratory incubated cores when compared with the field incubated cores in the fall and spring [see Appendix E] as was the case for ammonium-N, which suggests that availability of P may not be strongly regulated by soil microorganisms.

P is strongly immobilized in the litter layer from these slash pile plantations (Holtz, Perry, Cropper, and Hendry, in press). P contents in decaying needles increase 0-50% over the original amount after 24 months, with needles in the older stands showing greater accumulations. Throughfall concentrations can barely account for the observed increases, so that lower layers in the forest floor and/or mineral soil must be the sources of mineralized P available for plant uptake.

Although relatively small net changes in P concentrations after the incubation period were found, which rarely occurred in the summer, there may still be significant amounts of P to actively plant uptake reduce. Net P concentrations increased between 0.8-1.1 (0.5-1.2) in the control, 0.4-1.7 (0.7-1.8) in the burned, and 0.0-0.0 mg P/kg-soil

(2.1-2.5 kg/ha-yr) in the fertilized plots collected in the summer from the 6- or 26-year-old plantation (Table A6.1).

According to Shale, Fisher, and Fritchett (in press), uptake of P by vegetation in the unfertilized stands ranged from 3.3-4.3 kg P/ha-yr in an 6- and 26-year-old plantations, respectively. If I assume 4-6 months in which net amounts of unperiodically bound P is released through microbial activity, and if the double acid extractable P actually coincides well with plant uptake (as has been suggested by Thomas and Pauline, 1973, and Ballard and Fritchett, 1981), then even control plots appear to supply sufficient amounts of P (4 to 6 kg P/ha-yr) for vegetation uptake (Table A6.15). For the 6-year-old plantation, which is more dependent on availability of nutrients from the soil than on internal recycling (Gädfu, Fisher, and Fritchett, in press) exp. is more uncertain, but P limited. On the other hand, the supplying capacity observed in the control plots, appears to be sufficient to sustain uptake rates of the clear stand (3.3 kg P/ha-yr) if no internal recycling occurred. Internal recycling is high, and P concentrations in tissue are low (Shale, Fisher, and Fritchett in press) in these stands, which suggests that all the extractable P may not be available for the vegetation (less than 50% of the total extractable P detected, was found in the bottom 10 cm), or that the net increases in concentrations at the end of the incubation period are not always as high as those observed in this investigation. If the site is burned or fertilized, additional P may become available (Table A6.21), but increases seem to be temporary unless treatments are repeated regularly (if large amounts of P are applied at once through fertilization, potential leaching losses may

Table A2, B1. Sumner calculated net P concentration changes (kg/ha-yr) on the 14- and 20-yr-old plantations for the various treatments assuming either 4 or 8 months of mineral activity per year.

	TREATMENTS					
	Control		Burned		P ₂ O ₅ fertilized	
	4 months d		4 months d		4 months d	
min.	2.6	3.4	2.8	4.2	12.4	18.4
max.	4.4	7.2	7.4	11.4	22.0	32.0
\bar{x}	4.2	6.2	6.2	7.8	17.2	25.8

be high). As mentioned ion exchange resin extractions reflected that as much as 70% of the total P detected in the P-fertilized cores was trapped by the resin.

Figure 44.1 gives the best current estimates P fluxes for the 20-year-old plantation when not fertilized with P. Similar values occur in the other two sites, except in the clearcut area where high leaching losses are expected as little or no vegetation uptake occurred.

It is important to observe that the uptake values obtained by Dells, Fisher and Brinkmann (in press) may represent rates for vegetation already adapted to low concentrations of nutrients. According to Chapin (1980), major adaptations of some vegetation to low nutrient availability include slow growth rates and reduced annual nutrient requirements. In Chapin's experiments, when low-nutrient-adapted forest species were supplied with P, they had a lower capacity for P absorption and were less efficient in producing biomass, i.e. they tended to store the extra P. Although some of these characteristics are largely genetic in nature, some phenotypic flexibility may exist in species, such as slash pine, where they may react selectively depending on the nutrient availability in the soil.

Conclusions

The following conclusions can be drawn from the results obtained in this experiment:

- 1) Extractable P concentrations before and after the incubation period were similar in all plantation ages in the non-P-fertilized plots.
- 2) Burning increased P concentrations but increased were small and temporary.



Figure A6.3. Estimated phosphorylation fluxes (by *in silico*) in an unfertilized 20-prime-old zebrafish plus plastation (Morris and Fritzsche, 1992, and 1993, Finner, and Fritzsche, in press).

- 2) Phosphorus fertilization increased availability of double acid extractable P, as expected, but also increased the amount of P trapped in the bottom resin to as much as 70% of the total P detected in the cores.

Net P increases were higher in the 1- and 20-year-old plantations, although the opposite occurred in the recent cleared site.

- 3) Phosphorus concentrations in the PE plots dropped very fast after the fertilization, especially in the cleared area, which suggests that leaching losses were high. If this is the case, small amounts of P at a time must be added to the soil to avoid such losses.
- 4) Little differences in P concentrations before and after the incubation period were observed between incubation methods, suggesting that soil aeration and moisture content apparently did not have a strong effect.
- 5) In the Fall and spring, P concentrations before and after the incubation period decreased. Interestingly, only a small increase was observed in the laboratory incubated cores when compared with the field incubated cores which suggests that other factors besides temperature are controlling this process. A relatively large decrease in P concentration after the incubation period was observed in the spring and especially high in the PE fertilized plots.
- 6) Net increases in extractable P after the incubation period are apparently sufficient to satisfy P uptake rates of the vegetation in the 1-year-old and 20-year-old plantations, although P stress may still be possible in the 8-year-old plantation, as vegetation

is less dependent on internal recycling and growth requirements are greater than older plantations.

- i). In the non-fertilised plots, bottom resin and deionised water analysis indicated that P present in soil solution was low (less than 10% of the total P detected in the cores, Appendix E). This suggests that mass flow transport of P to the roots may be a limiting factor in vegetation uptake.

APPENDIX B

EFFECTS OF AIR-DRYING AND REWETTING A RESIN-BINDING EXCHANGE METER

Introduction

Ion exchange resins (IER) have recently been used to measure forest soil nitrogen (N) availability (Hinsley and Matson, 1980) and N leaching (Schaefer, 1980) with very encouraging results. In addition, P extraction from the anion portion of the resin has been used as P availability index (Barr et al., 1980; Olson and Sommers, 1982). This method offers the advantage of being sensitive to an site conditions, when used in field experiments, without producing pH changes or other chemical alterations in the soil.

A key factor to their effective use is the quantitative and reproducible extraction of N ions (NO_3^- , NH_4^+) from the resin complex. Several tests have been performed on the N extraction efficiency of IER (Schaefer, 1980; Kirk and Hinsley, 1984) and on the effect of time on the amount of N retained by the resin (Schaefer, 1983; Hinsley, in press). These studies have concluded that extraction efficiencies for the ammonium and nitrate-N are high (80 to 90%) and that no transformations occur even after 22 weeks of storage.

On the other hand, no studies have been made on the impact of drying and wetting cycles, a potentially frequent occurrence in the surface of forest soils, and on the ability of the resin to capture and/or retain N species. It is known that physical damage does

occur when the resin is deliquesced [B. W. Tanaka, *Japan Chem. Rev.*, pers. comm., 1993].

The purpose of the present investigation was to study the effects of moisture losses and wetting cycles on the IIR properties.

Methods

A self-indicating, certified research grade, mixed-bed IIR (Range 1-200, Fisher Scientific Co.) was used. Properties are presented in Table I. The experiment was divided in two parts.

A) Ten flasks each containing 5 g (wet weight) of the IIR were allowed to dry to different moisture contents (ranging from about 38 to 99%, by weight), rewetted and air-dried again. After the second cycle, 30 ml of ammonium nitrate (5.1 mg/ml-5.5 mg N/ml) were added to 5 g resin and mixed for 1 hr (a very high concentration was added to test the ability of the resin to retain high levels of N after wetting-drying cycles). The solution was then filtered with a Whatman #42 filter paper and analyzed for ammonium-N using the classic distillation procedure [Brown, 1985] and nitrate-N using the Technicon Autoanalyzer II [Technicon Industrial Systems, 1993].

This part tested whether or not the ability of the resin to trap added NO_3^- and NO_2^- is affected by prior drying-wetting cycles.

B) Ten flasks each with 5 g (wet weight) of the IIR were mixed with 30 ml of ammonium nitrate (5.1 mg/ml-5.5 mg N/ml) for 1 hr. Excess solution was drained and discarded (it was assumed all the ammonium nitrate was trapped by the resin). The resin was then allowed to dry to different moisture contents ranging from 38 to 40%, by weight. Resin was rewetted by pouring 30 ml of deionized water into the resin sitting in a funnel with a Whatman #42 filter paper. Excess water was then

analysed in the steam distillation to determine ammonium- and nitrate-N. Finally, the washed resin was air dried again and retested for a second time as before. Again nitrogen was not extracted with HCl at the end of the experiment.

This part tested whether or not added ammonium and nitrate were released after a cycle of drying and wetting.

Results and Discussion

Data obtained from the first part of the experiment, i.e. air drying the resin without N, are shown in Table 43.1. In general, the drier the resin the less ammonium and nitrate was trapped (more was detected in the filtrate) until a resin weight of approximately 8 g was reached. At this point, N concentrations in the filtrate dropped, but began rising again as the resin lost more water.

These results appear to indicate that some of the exchange sites of the resin were destroyed by the treatment, but when a certain wetting content was reached some of the ammonium and nitrate ions may have been absorbed by the dried beads. This suggests that rather than a chemical interaction with the ion exchange sites, ions were physically trapped inside the beads. As the resin got drier, more exchange sites were destroyed, but no more available space inside the bead was created, so that the concentrations of nitrogen increased again.

In addition, it was found that the control's produced some contamination which led to overestimation of the ammonium-N in steam distillation procedure. This interference may have been due to the presence of the quarternary amine, which forms part of the cation group of the resin, as it reacted with the naphthol reagent.

Table III. Beads weights after the first (I) and second (II) air-drying cycles, and the concentrations of amoniac- and nitrate-N in the filtrate solution of the added ammonium nitrate (not trapped by resin).

I mg/g	II mg/g	$\text{NH}_4\text{-N}$ mg/l	$\text{NO}_3\text{-N}$ mg/l
5.0 ^a	5.0	5.3	0.0
5.0 ^a	5.0	5.7	0.0
5.0	5.0	5.3	0.2
5.0	5.0	5.4	0.3
5.0	5.0	10.0	0.0
5.0	5.0	15.4	1.3
7.0	5.0	12.6	1.4
10.0	5.0	2.4	0.0
15.0	5.0	4.2	0.4
5.0	5.0	10.4	0.0
5.0	5.0	5.0	1.3

^a Controls, no N added, not air dried.

Results from Part II are presented in Table A6.2. After the first cycle there was an obvious increase in the nitrate-N released by the resin as it became drier. After the second cycle, this tendency was not observed although more nitrate-N was detected. Part of this nitrate-N may have been released from the anion exchange sites as a result of a concentration difference between the desorbed water and the resin beads. Very little of the ammonium-N was released. Again some contamination was observed in the controls.

Conclusions

There is an indication that when the total moisture content of the resin is below 50%, resin ion exchange properties are affected. Higher concentrations of N were detected in the filtrate solutions after the drying and wetting cycles, supporting that some N may not be trapped, or once N is in the resin, it may be released, [especially in the case of nitrate].

Under field conditions the resin is not normally expected to lose high amounts of moisture unless it is placed uncovered near the surface, or if the soil drastically dries for long periods of time. In addition, the amounts of N not trapped or released by the resin after the treatment, were very low relative to the high concentrations of N added to the resin. In the field, much lower concentrations usually occur, so that if some ion exchange sites of the resin are damaged, there should still be adequate sites available to capture and retain N in the soil solution.

Table A6.2. Bead weights and ammonium and nitrate-N concentrations in the deliveted water wash after the first (I) and second (II) air-drying cycles.

No	I		No	II	
	NO ₃ -N	NO ₂ -N		NO ₃ -N	NO ₂ -N
	----- mg/l -----			----- mg/l -----	
6.19	8.1	0.7	6.0	8.4	0.8
6.19	7.8	0.6	6.1	8.7	0.9
7.4	1.3	0.3	6.7	8.8	0.9
7.1	1.3	1.1	6.3	8.8	4.1
6.3	4.2	3.6	6.9	8.3	4.9
6.9	4.8	3.6	6.4	8.3	4.1
6.2	7.6	4.7	6.6	8.8	3.8
6.1	7.6	4.9	6.8	8.3	3.8
6.6	8.9	4.9	6.6	8.7	4.3
6.8	8.9	5.2	6.8	8.3	3.8
6.8	7.0	5.1	6.8	7.7	4.3

*= controls, air-dried, no N added.

APPENDIX I
CALIBRATION CURVE ON THE ION EXCHANGE RESIN

Introduction

No interference was found when americium-241 extracted from a mixed-bed ion exchange resin (IX) was analyzed with the Technicon Autoanalyzer II. When concentrations of americium-241 were low, color did not develop. It was found that by changing the concentration of sodium hypochlorite from 4 to 8 ml/100 ml and sodium citrate/ascorbate from 0.10 to 0.25 g/1000 ml in the Technicon procedure, and by eluting the sample, americium-241 could be detected.

The purpose of this section is to describe the calibration a relationship between the amount of americium-241 detected by the Technicon and that actually added to the IX. This relationship was later used to determine the concentration of americium-241 trapped in the resin coming from the field located cores.

Methods

Two flasks each containing an equivalent of 2 g. of air dry IX were mixed for 3 hr with 50 ml of a solution containing several concentrations of americium chloride (3-10 mg/l). After mixing, the solution was filtered with a Whatman #42 filter paper. The distilled water was discarded ensuring that all the R was trapped by the IX.

The IX with the added R was air dried for 3 days and then mixed with 50 ml of a 2N HCl solution for 3 hr. The eluate was filtered as before and analyzed with the modified Technicon procedure. Before the

analysis, the samples were diluted 2.5, 5 and 40 times. Levels of methionine-R extracted from the IIR were compared with the actual amount added.

Results

Results obtained from this experiment are shown in Figs. 36, 37. In all dilution cases (e.g., 2.5, 5, and 40 times), the relationship between the methionine-R levels extracted from the IIR and the actual R amounts added were linear and with very high r^2 's. The equations were

$$P_{2.5} = 0.158 + 0.380 x; r^2 = 0.997$$

$$P_5 = 0.167 + 0.515 x; r^2 = 0.998$$

$$P_{40} = 0.950 + 1.877 x; r^2 = 0.997$$

where

y = methionine-R concentration extracted from the IIR, and

x = methionine-R concentration actually added to the IIR

For the 2.5 and 5 times dilution factors, R extracted from the IIR was lower than the amount added, but when sample was diluted 40 times, R extracted appear to be higher than the amounts added.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy


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